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FOREWORD

Starting a new journal is always a difficult and controversial undertaking. There are so many existing publications that most research workers are already overwhelmed by them, and the cost of maintaining an adequate library is becoming excessive. On the other hand the results of painstaking original work may have to wait too long before they are published, and so are not circulated rapidly enough among those concerned with similar problems, because many editors cannot guarantee speedy publication. We therefore believe that a journal with a relatively wide scope is called for, and we hope that the flow of ideas and results will be stimulated by this new publication.

Up to the present the contacts between experimental and applied entomology have been too restricted. However, many of the classical discoveries in insect physiology and ecology have been made by workers in applied entomology (a fact of which the textbooks bear ample witness) and applied entomologists have profited greatly from the study of experimental entomology for its own sake. Problems concerning the resistance of crop plants to insects, diapause, phenology, biological control and resistance to insecticides are among the important questions relating to insect control which are being successfully attacked bij experimental workers the world over.

This journal offers a meeting place to both parties, and it is hoped that it will cause mutual stimulation in these two fields in which interest is growing. It will deal with research on other land arthropods as well as insects, thus papers concerning millipedes and isopods may be accepted, but work on aquatic crustaceae is excluded. All papers submitted should have an experimental basis ; purely descriptive ecology is therefore not acceptable.

We are glad to be able to publish in the first number most of the main papers read at a Symposium on "Insect and Foodplant" held at Wageningen, the Netherlands, in 1957. Though the journal is intended to have a wider scope than this first number might suggest, this series of papers by specialists in biochemistry, insect physiology, ecology, behaviour and plant breeding has many features which we hope will continue to be found in future numbers. The Symposium was a successful attempt to secure coordination between specialists in many fields, and also to encourage the exchange of information between scientists of many nationalities. The publication of the results of this experiment may emphasise one of the main purposes at which we aim.

The establishment of this journal would not have been possible without the initiative of the "Nederlandsche Entomologische Vereeniging" and the financial help of the "Uyttenbogaard-Eliasen Stichting". We are grateful for their generous support.

THE EDITORS

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ÜBER DEN FRASSTEST AN LEPTINOTARSA-RESISTENTEN KARTOFFELN

VON

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Die Testung von Kartoffelhybriden auf Resistenz gegen den Kartoffelkäfer bereitet Schwierigkeiten, da die Ergebnisse schwanken und die Ursache nicht geklärt ist. Trotz ungünstigem Erbgang wurden auf der Basis von *Solanum chacoense* beachtliche züchterische Erfolge erzielt.

Als ich vor 16 Jahren mit der Resistenzzüchtung gegen den Kartoffelkäfer (*Leptinotarsa decemlineata* Say.) begann, hatte ich glücklicherweise keine ausreichende Vorstellung von den Schwierigkeiten, die mich einmal von der Seite der Pflanze, aber nicht minder vom Insekt erwarteten. Sonst hätte ich vielleicht nicht den Mut gehabt, an die mir gestellte Aufgabe heranzugehen. Damals stand die mexikanische Wildkartoffel *Solanum demissum* im Mittelpunkt des züchterischen Interesses. MÜLLER und SELLKE (1941) hatten die Resistenz von Tuberosum-Hybriden dieser Wildart untersucht und in späteren Generationen nur anfällige Bastarde gefunden. Mit diesen Ergebnissen wurde die Resistenzzüchtung auf der Grundlage von *Solanum demissum* problematisch. Sie ist es geblieben bis zum heutigen Tag.

Das war der Grund, daß unser Institut sich nicht lange bei den Demissa aufhielt, sondern der Käferresistenz der Commersoniana zuwandte. Das erste Glied, das uns durch hervorragende Resistenz auffiel, war *Solanum chacoense* (TORKA 1943). Aber auch bei anderen Species dieser Serie ist Käferresistenz weit verbreitet; jedoch treten neben resistenten Pflanzen mehr oder weniger anfällige auf (TORKA 1954). Ihre geographische Verbreitung erstreckt sich auf die atlantischen südamerikanischen Staaten, vor allem Argentinien, Uruguay und Paraguay. Die Resistenz des *Solanum chacoense* läßt sich folgendermaßen charakterisieren: Im Gegensatz zu *Solanum demissum,* dessen Wirksamkeit sich vorwiegend auf die Larven erstreckt (SCHAPER 1939), werden hochresistente Chacoensepflanzen weder von der Larve noch vom Käfer gefressen (TORKA 1949). Bei ausschließlicher Fütterung mit Chacoenselaub müssen beide praktisch verhungern. Die Resistenz wird nach neuesten Untersuchungen von KUHN (1957) durch bisher unbekannte, wasserlösliche Alkaloide hervorgerufen. Diese wirken wahrscheinlich als „repellents" und nicht toxisch.

Der gegenwärtige Stand unserer züchterischen Arbeit ist folgender: Durch Inzucht und strenge Selektion der widerstandsfähigsten Pflanzen wurde *Solanum chacoense* homozygot resistent. Colchicin-Behandlung verdoppelte deren 24 Chromosomen, wonach Kreuzungen mit 48-chromosomigem *Solanum tuberosum* ohne

Schwierigkeit gelangen. Diese Hybriden unterscheiden sich in ihrer Käferresistenz von entsprechenden Kreuzungen mit *Solanum demissum* durch einen anderen Erbgang. Diese Unterschiede sind in Tabelle I gegenübergestellt.

TABELLE I

Käferresistenz der Hybriden von Solanum demissum und Solanum chacoense

1. *Solanum demissum-Hybriden*			2. *Solanum chacoense-Hybriden*		
Kreuzungsgeneration	Eltern	larvenresist. Pflanzen in $^0/_0$	Kreuzungsgeneration	Eltern	larven u. käferresist. Pfl. in $^0/_0$
F_1	d × t	~ 20 %	F_1	ch × t	0 %
F_2	(d × t) × s	fast 0 %	F_2	(ch × t) × S	1,5—5 %
F'_2	(d × t) × t	2—4 %	F'_2	(ch × t) × t	0 %
	F'_2 × S	fast 0 %		F'_2 × S	~ 2 %
				F'_2 × res. F_2	2—15 %
				res.F_x × res.F_x	> 20 %

Erklärung der Abkürzungen:

d = Solanum demissum; t = Solanum tuberosum; ch = Solanum chacoense; S = Selbstung; F_1 = 1. Kreuzungsgeneration; F_2 = 2. Kreuzungsgeneration; F_2 = Tuberosum-Rückkreuzung.

Bei den Demissum-Hybriden gewinnt man den Eindruck, daß die Wildspeciesresistenz schon in den ersten Generationen immer mehr verdünnt wird und schnell erlischt. Bei Chacoense-Hybriden liegen die Verhältnisse anders. In der F_2 tritt die Resistenz wieder in Erscheinung, nachdem sie in der F_1 fehlte. Durch sorgsam gelenkte Kreuzungen gelingt es langsam, die Leistungsfähigkeit der Zuchtstämme zu verbessern, ohne daß die Resistenz verloren geht (TORKA 1950). Bei dominantem Erbgang wäre die Arbeit wesentlich einfacher. Schon in der F'_4 wären sortenähnliche resistente Kartoffeln zu erwarten. So aber mußten in den zurückliegenden Jahren viele Tausende von Pflanzen einzeln auf Käferfestigkeit geprüft werden, ehe einige resistente Klone übrig blieben. In der F_2 waren beispielsweise von 100 Sämlingen 1—5 Pflanzen resistent. Außerdem bringt nicht jeder Wildkartoffel-Bastard Knollen hervor; manche fallen vor der Ernte Krankheiten zum Opfer. Die zahlenmäßig kleine resistente F_2 mit vielen Wildmerkmalen muß zur Leistungssteigerung mit *Solanum tuberosum* rückgekreuzt werden (TOXOPEUS (1952) nennt diesen Vorgang „tuberisation"). Die so erzielte Leistungssteigerung macht die Pflanzen käferanfällig, und die Resistenz muß durch Selbstung, Geschwisterkreuzung oder Kombination mit einem resistenten Bastard wiedergewonnen werden. Das gibt Riesenzahlen von Sämlingen, die alle einzeln auf Resistenz geprüft werden müssen. Die anfangs geringe Zahl der übrigbleibenden Pflanzen steigt stetig an, und die Basis der Resistenzzüchtung wird immer breiter. Die Chance für die resistente Kartoffelsorte wird durch Leistungsverbesserung immer größer. Soweit sind gegenwärtig die Arbeiten der Käferstation des Max-Planck-Instituts für Züchtungsforschung gediehen; aber bis dahin mußte ein weiter Weg zurückgelegt werden.

Hatte die Übertragung der Resistenzgene in die Kulturkartoffel grosse Schwierigkeiten bereitet, so wurde unsere Arbeit außerdem durch das Verhalten des Kartoffelkäfers erschwert. Wer noch nie derartige Resistenzprüfungen durchgeführt hat, kann sich wahrscheinlich nicht vorstellen, wieviel Zeit und Erfahrung nötig sind, um eindeutig von einer Pflanze sagen zu können: „diese ist resistent".

Wird eine Chacoense-Hybride reichlich gefressen, so ist sie anfällig, und der Fall ist eindeutig. Wird sie nicht gefressen, so kann sie resistent sein, aber in vielen Fällen ist sie erfahrungsgemäß anfällig, was sich häufig erst im nächsten Versuchsjahr erweist. Bei manchen Zuchtklonen wird der Irrtum sogar erst im dritten Jahr erkannt. Das liegt an der Unzuverlässigkeit aller Fraßteste. Aber warum eine anfällige Kartoffel manchmal nicht gefressen wird, ist unbekannt. Über die Durchführung der Teste folgendes: Anfangs fütterten wir L_1-Larven im Hygrostaten mit dem Laub unserer Klone bis zur dritten Häutung und bewerteten Fraß und Mortalität. Der Futterwechsel und die Registrierung der überlebenden Larven und ihrer Entwicklung ermöglichten wegen des großen Arbeitsaufwands nur eine relativ kleine Zahl von Prüfungen, die den züchterischen Anforderungen nicht genügten. Darum gingen wir später dazu über, uns auf Fraßbewertungen nach 24- oder 48-stündiger Fütterung zu beschränken. Dabei legen wir großen Wert auf gleichbleibende optimale Bedingungen für die Larven wie hohe Temperatur und Luftfeuchtigkeit und frisches, junges Futterlaub (GRISON 1951). Alle Versuche werden im Brutschrank durchgeführt. Da für jede Wiederholung nur ein Fiederblättchen benötigt wird, können Sämlinge bereits im ersten Jahr und verhältnismäßig jung getestet werden, wodurch sich die Zahl der zu pflegenden Pflanzen schnell verringert, da alle gefressenen sofort ausgeschieden werden. Wir haben die Durchführung derart mechanisiert, daß zwei Personen ohne übermäßige Anstrengungen 800 Teste am Tage durchführen können. In Vergleichsprüfungen fielen diese Fraßversuche im Thermostaten nicht schlechter aus als die Larvenaufzucht im Hygrostaten. Aber beide Ergebnisse lassen zu wünschen übrig.

Einige Beispiele sollen das Gesagte unterbauen. Es gibt in unserem Zuchtmaterial Sämlinge oder Klone, die einen ganzen Sommer lang nicht gefressen werden und auf denen sich die Larven nicht entwickeln. Dabei ist es gleichgültig, ob ich junge oder ältere Blättchen füttere, ob ich eine oder 20 Wiederholungen anstelle. Drei Wochen später wird das gleiche Ergebnis erzielt, alle Wiederholungen sind ohne Fraßspuren, die Larven meiden das Blatt und kriechen suchend umher. Das kann sich im Laufe eines Sommers wiederholen, und der Versuchsansteller gewinnt den Eindruck, daß es sich um eine hochresistente Pflanze handelt. Im kommenden Jahr fressen die Larven reichlich an dem gleichen Klon. Mit der größeren Zahl von Wiederholungen oder aufgesetzten Larven läßt sich dieser Versuchsfehler nicht vermeiden. Ganze Saatnummern, die im ersten Versuchsjahr als besonders hoffnungsreich angesehen wurden, versagten im nächsten Sommer. In einem Jahr narrten uns eine größere Zahl von F_1-Bastarden, die einfach nicht gefressen wurden. Wir glaubten, unsere bisherigen Erfahrungen über die Anfälligkeit der ersten Kreuzungsgeneration korrigieren zu müssen — da war sie im nächsten Sommer ausnahmslos anfällig.

Der geschilderte Tatbestand bringt begreiflicherweise eine ständige Unsicherheit in unsere Arbeit, sodaß wir uns bei dieser Prüfungsmethode nicht wohlfühlen können. Wir besitzen aber vorläufig keine bessere, wenn wir auch noch immer die Hoffnung haben, eines Tages den Resistenzstoff quantitativ bestimmen zu können mit Hilfe einer einfach durchzuführenden chemischen Reaktion.

Eine Erklärung für dieses rätselhafte Verhalten der Larven zu finden, ist bisher nicht gelückt. Dabei erhebt sich die Frage, ob die Larven der Käferpopulationen unserer Felder sich einheitlich auf der Wildkartoffel verhalten oder Biotypen mit größerer Anpassungsfähigkeit vorhanden sind. Beobachtungen über Unterschiede liegen tatsächlich bei uns vor. Es gibt einzelne Larven, die in bescheidenem Umfang Chacoenselaub zu fressen vermögen, und gelegentlich entwickelt sich daraus ein Käfer. Aber diese Beobachtungen erklären nicht das geschilderte wechselvolle Verhalten. Dann dürften nur einzelne Wiederholungen nicht gefressen werden — es sind aber ganze Serien von Larven, die aus verschiedenen Gelegen stammen und sich doch einheitlich benehmen, d. h. zeitweise fressen sie nicht, zum anderen Zeitpunkt schmeckt ihnen das gleiche Futter offensichtlich. Aus diesem Grunde suchen wir die Erklärung in Veränderungen der Pflanze, beispielsweise modifikativen Unterschieden ihrer Zusammensetzung. Es besteht die Möglichkeit, daß ein Vergällungsstoff nur in der Epidermis lokalisiert ist; denn sobald diese nach langer Hungerzeit von der ersten Larve angenagt ist, beginnen die übrigen Larven an der gleichen Stelle zu fressen.

Wir sind auf dem Rosenhof in der günstigen Lage, dank der starken Verseuchung unseres Gebietes mit *Leptinotarsa*, Freilandprüfungen durchführen zu können. Unter optimalen Bedingungen für das Insekt sind Fraßversuche sehr eindrucksvoll und zur Bestätigung der im Labor gewonnenen Ergebnisse unerläßlich. Aber die optimalen Bedingungen sind in Mitteleuropa erfahrungsgemäß nur in wenigen Sommern vorhanden. Im Frühjahr wird unser Versuchsfeld mit anfälligen und resistenten Hybriden bepflanzt. Schon Ende Mai legen die zufliegenden Käferweibchen an alle jungen Stauden reichlich viele Eigelege. Ist das Frühjahr warm und sonnig, so können die Pflanzen von den sich darauf entwickelnden Larven binnen 20 Tagen kahlgefressen sein, dann sind sie anfällig. Auf resistenten Hybriden gehen die Larven bald zugrunde, und der Fraßschaden ist gering oder fehlt ganz. Mehrfache Wiederholung des Versuchs steigert die Zuverlässigkeit des Ergebnisses — ist doch manchmal der Befall nicht auf allen Teilen des Feldes gleich stark.

Bekanntlich waren die letzten Sommer ungewöhnlich kühl und verregnet, was für unseren Feldversuch üble Folgen hatte. Vielfach gingen die Eigelege zugrunde, bevor die Larven schlüpften, oder diese erstarrten und wurden vom Regen abgespült. So entsprach der Fraßschaden nicht dem gewohnten Bild, und in vielen Fällen war eine Beurteilung der Resistenz nicht möglich. In manchen Sommern beobachteten wir im Juni einen plötzlichen Rückgang der Larven, diese hörten auf zu fressen und sahen krank aus und starben bald. So wird durch Witterungseinflüsse und Krankheiten die Zuverlässigkeit dieser Prüfung stark beeinträchtigt. Zuchtklone, die im einen Jahr keinen nennenswerten Fraßschaden aufwiesen, enttäuschten im folgenden Sommer durch Kahlfraß.

Wenn die 1. Larvengeneration keine befriedigenden Fraßunterschiede zwischen resistenten und anfälligen Hybriden hervorbringt, bleibt die Hoffnung auf günstigere Verhältnisse beim Auftreten der Jungkäfer und 2. Larvengeneration im August. Frühreife Stämme sind zu diesem Zeitpunkt häufig schon welk, aber an späten sind Fraßunterschiede oft besser zu sehen als im Frühsommer. Aber in manchen besonders ungünstigen Jahren sind in beiden Käfergenerationen keine Befallsunterschiede feststellbar, und der Feldversuch ist umsonst angelegt.

Zum Abschluß soll eine Frage gestreift werden, die für die Resistenzzüchtung von außerordentlicher Wichtigkeit ist und auch mit den Larventesten in Verbindung steht. Chacoenselaub verursacht auf der menschlichen Zunge ein starkes Brennen und widerlichen Bittergeschmack. So war zu befürchten, daß der Käfer bzw. die Larve ähnliche Geschmacksempfindungen hat und die Resistenz mit Bitterkeit gekoppelt ist. Die Knollen unserer älteren resistenten Hybriden wiesen tatsächlich einen ebenso widerlichen Geschmack auf wie Chacoenselaub und waren ungenießbar. Darauf hat im Jahre 1953 Prof. KRAUT vom Max-Planck-Institut für Ernährungsphysiologie in Dortmund freundlicherweise mit den gleichen Knollen Fütterungsversuche an Ratten durchgeführt. Erwachsene Ratten starben ausnahmslos nach 1—3 tägiger Fütterung mit Kartoffelbrei dieser resistenten Hybriden, während die Kontrollen gesund blieben.

Inzwischen ergaben planmäßige Geschmacksprüfungen an den Knollen unseres resistenten Zuchtmaterials große Unterschiede. Die resistenten Klone umfassen sowohl neutral als auch bitter schmeckende Stämme, sodaß das Geschmacksproblem verhältnismäßig schnell durch Selektion zu lösen war. Außerdem isolierten KUHN & LÖW (1955) aus *Solanum chacoense* sechs verschiedene nicht larvenwirksame Alkaloide, die wahrscheinlich den widerlichen Geschmack der Wildkartoffel hervorrufen und außerdem giftig sind. An diesen Alkaloiden und nicht am Resistenzstoff dürften die Ratten ums Leben gekommen sein. Eine Wiederholung der Versuche mit solaninarmen, käferresistenten Zuchtstämmen steht noch aus.

RÉSUMÉ

Les *Commersoniana,* Pommes de terre sauvages, sont des géniteurs résistants au Doryphore. Les premières générations de croisement avec *Solanum tuberosum* n'ont que peu d'hybrides résistants, mais la résistance est constante.

Les tests biologiques avec le Doryphore sont satisfaisants pour la sélection des Pommes de terre résistantes. Mais il faut les répéter plusieurs années.

Il y a des différences parmi les insectes. Quelques-uns peuvent consommer un peu de feuillage de *Solanum chacoense,* Pomme de terre sauvage résistante au Doryphore. Mais les hybrides sélectionnés restent résistants en plein air.

Quelques hybrides sensibles ne sont pas toujours mangés par le Doryphore. Peut-être ces plantes contiennent-elles une substance variable répulsive qui n'est pas identique avec le principe de résistance.

Les tubercules de *Solanum chacoense* ont un goût amer résultant de six alcaloïdes toxiques. Nous avons trouvé des hybrides présentant une résistance constante et un goût agréable.

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DISKUSSION

D. HILLE RIS LAMBERS hält die Züchtung toleranter Kartoffelstämme, die nicht in jedem Jahr gleich widerstandsfähig gegen den Kartoffelkäfer sind, für einen beachtlichen Fortschritt.

J. DE WILDE berichtet über eigene Erfahrungen über den Wettereinfluß und physiologisch-chemische Veränderungen des Futterlaubes. Diese lassen sich experimentell während der kurzen Dauer der Fraßteste nicht ergründen.

K. SCHREIBER betont ebenfalls den großen Einfluß des physiologischen Zustandes der Futterpflanzen. Nach seinen toxikologischen Untersuchungen mit weißen Mäusen (perorale Applikation) liegt die Dosis letalis min. für 3 Alkaloide ungefähr in der gleichen Höhe und beträgt für Solanin, Chaconin und Demissin 0,7—0,8 mg Alkaloid/g Maus.

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L'IMMUNITÉ CHEZ LES SOLANACÉES À L'ÉGARD DU DORYPHORE

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RÉSUMÉ

Dans l'étude des forces de la nature pouvant être, au besoin, mises à profit pour réduire les dégâts du Doryphore (*Leptinotarsa decemlineata* Say), trois voies principales de recherches étaient à suivre.

Elles furent abordées en 1927, lorsqu'il apparût que l'on ne pourrait plus éteindre le foyer de l'insecte découvert en 1924 dans le sud-ouest de la France.

Ces trois voies concernaient:

- l'action sur le Doryphore de facteurs a-biotiques (climat et parfois sol);
- l'action sur le Doryphore d'ennemis naturels, entomophages et entomophytes;
- enfin, l'action sur l'insecte de certains facteurs propres d'immunité pouvant être présentés par les végétaux qu'il attaque ou pourrait attaquer.

En suivant la première orientation, un certain nombre d'utilisations en quelque sorte „passives", ont eu lieu. En particulier elles portent sur la mise à profit de l'action freinatrice du climat au printemps découlant du fait que insecte et pommes de terre n'ont pas les mêmes seuils inférieurs d'exigences thermiques, aussi peut-on l'utiliser pour certains déphasages pouvant donner des effets très marqués.

Ils permettent l'établissement de cultures de primeurs pour les pommes de terre, pratiquement sans traitements chimiques, surtout lorsqu'on prend soin de veiller dans le secteur où on les établit, à l'absence de cultures de saison de pommes de terre et de plantations d'aubergines (*Solanum melongena* L.).

Dans la voie de la lutte biologique au sens restreint habituel du terme, les premières études — études de base — se sont déroulées surtout de 1928 à 1939. Les travaux vont être repris par la Commission Internationale de lutte biologique, Commission récemment crée et qui comporte un groupe de travail sur les ennemis naturels du Doryphore présidé par le Dr. FRANZ de Darmstadt. Ce groupe a tenu sa première réunion cet hiver, à Gembloux, et a fixé deux orientations principales pour ses efforts: avoir le minimum d'action fâcheuse des traitements insecticides sur le jeu de la lutte biologique naturelle; si possible, avoir à l'avenir en Europe en appoint de nos entomophages indigènes, d'autres espèces venant, elles, du pays d'origine du Doryphore.

La troisième voie comprenait les études d'immunologie et leur mise à profit dans la recherche de plantes cultivées si possible peu sensibles aux attaques, voir même résistantes.

Le rappel ici des trois grandes orientations d'étude n'a pas qu'une seule valeur historique.

Il permet de préciser qu'il apparaît de plus en plus nettement que des rapports d'interdépendance doivent exister entre les trois catégories de travaux cités si nous cherchons à obtenir la limitation naturelle la plus marquée des dégâts du Doryphore.

La solution écologique de lutte avec le minimum d'interventions chimiques onéreuses se montre comme devant être le plus souvent le fait d'une synthèse bien équilibrée entre ces éléments divers et non résulter de l'action exclusive d'un seul d'entre eux.

En d'autres termes, le problème des plantes échappant d'elles même aux dégâts sérieux du Doryphore, sera plus aisé à résoudre pratiquement si on envisage parallèlement l'action au mieux des facteurs abiotiques et de la lutte biologique.

En conclusion: d'abord tout progrès en lutte biologique intéresse indirectement nos travaux et réciproquement; ensuite, dans les études d'immunologie, une part de liaison pour information régulière s'impose avec divers travaux zoologiques; les zoologistes peuvent seuls l'établir avec les interprétations appropriées, et ce fait apporte un élément de plus montrant que pour l'immunologie et ses applications, le travail de base revient obligatoirement à des équipes mixtes: généticiens-zoologistes.

Abordons maintenant, en le détaillant plus, à titre documentaire, un secteur restreint: les affinités existant entre le Doryphore et les Solanacées diverses, notamment les non tubérifères.

Les recherches dans cette voie ont été abordées en France peu après l'arrivée du Doryphore, mais elles furent amplifiées à partir de 1930, surtout entre 1934 et 1939 lorsque dans le cadre de notre Laboratoire de campagne d'Ahun (France), des équipes venant d'autres pays — Allemagne, Pologne — s'ajoutèrent à la nôtre.

Certaines plantes furent étudiées à nouveau après 1945, non exclusivement en France cette fois. Leur travail principal a porté alors sur des Solanacées non tuberifères; de ce fait il sera développé dans d'autres notes. Toutefois, sur les Solanacées non tuberifères pendant cette période, citons d'intéressantes observations effectuées les unes aux Pays-bas, sur les *Hyosciamus* en particulier, d'autres en Espagne, France et Italie, sur *S. melongena* et les *Lycopersicum.*

Les travaux d'immunologie conduits en France avant 1940, sont surtout l'oeuvre de FEYTAUD, puis de TROUVELOT, DUSSY, THENARD, GRISON et BUSNEL, du côté francais; de MULLER-BOHME, SELLKE et SCHAPER, du côté allemand; de Mlle BOCZKOWSKA pour la Pologne.

Lorsqu'on regarde les relevés d'attaques par le Doryphore effectués sur des plantations expérimentales de Solanacées diverses, les uns répétés pendant plusieurs années en un même lieu, les autres réalisées dans des ambiances variées la même année, deux remarques principales s'imposent rapidement.

En premier lieu, il n'existe pas d'une part des plantes nettement attaquées par le Doryphore et d'autres échappant à ces attaques, c'est à dire deux catégories bien tranchées de végétaux. En fait, on note très nettement un échelonnement continu dans les degrés d'attaques, donc d'affinités, depuis les Solanacées dont les peuplements sont toujours très rapidement anéantis, conduisant en même temps à de fortes pullulations d'insectes, jusqu'à celles qui pratiquement ne sont pas attaquées et n'assurent aucune vie au Doryphore.

Ensuite, pour tous les végétaux, mais à des degrés assez variables selon les espèces ou formes, s'il y a possibilité d'attaque, celle-ci présente des oscillations dans son niveau, oscillations qui se révèlent en rapport avec l'ambiance écologique et avec l'état physiologique de la plante hôte, au moment de l'attaque: organe consommé et son âge; développement végétatif de la plante; sol et fumure; insolation reçue;...

En présence de faits aussi complexes on voit le manque de portée que peuvent avoir les observations ne portant pas sur des relevés continuels minutieux et se bornant, comme certaines personnes l'indiquent parfois, à laisser une attaque de Doryphore se produire sur des petits groupes de plantes situés les uns près des autres, puis noter en fin de travail ceux qui seuls subsistent. De tels relevés non seulement sont peu instructifs, mais encore se montrent souvent trompeurs; ils peuvent éliminer des nuances dans les modalités d'attaque, des effets marqués mais avec délais, dans les développements, comme on pourra s'en rendre compte par la suite.

Pour analyser le jeu, le mécanisme d'éléments d'immunité présentés par des plantes, l'observation conduit à examiner la serie suivante des rapports insectes-plantes hôtes.

Les rapports essentiels *imago*-plantes apparaissent de trois ordres. D'abord, les éléments „d'affinité" réglant l'arrivée, le stationnement, de l'*imago* sur des plantes hôtes. Une attraction, surtout à distance, à notre connaissance n'est pas démontrée. Par contre, des retentions existent, en rapport avec le micro-climat, causées par tel port et nature du feuillage, surtout avec des influences tactiles (notamment par l'intermédiaire des palpes) et gustatives. Sur ces dernières influences un parallèle serait à rechercher avec les observations récentes japonaises, vérifiées en France, sur le rôle limitateur très accentué du contact des palpes avec les feuilles, l'attaque de plantes, sans lui, serait beaucoup plus variée pour le Ver à soie, nombreuses plantes assurant la croissance aux chenilles privées de leurs palpes.

Pour les *imagos* du Doryphore, des plantes se sont montrées nettement retentives; telles sont (la classification suivante concerne des végétaux ayant vécu en plein soleil): *S. melongena* et *S. dulcamara:* des Solanées epineuses comme *S. rostratum* certaines tubérifères comme *S. edinense, S. andigenum, S. maglia, S. demissum*; enfin les *Petunia,* les *Salpiglossis.*

Après des échelons intermédiaires progressifs offerts par exemple par *S. heterodoxum*, *S. sisymbriifolium*, les *Nicotiana alba, sandere*, nombreux *Lycopersicum*, dans l'échelle décroissante des plantes retentives, nous rencontrions comme termes inférieurs, nombreuses formes du *S. nigrum* les *S. rantonetti, aculeatissimum.*

La seconde phase de comportement à examiner dans l'interaction Doryphore-plantes hôtes est la prise du premier aliment par l'imago, l'intensité dans l'attaque au départ puis la persistance de cette attaque. Le jeu de cet ensemble de facteurs de comportement, donne un classement des plantes à peu près analogue au précédent mais certaines différences intéressantes apparaissent. Sur des plantes comme *S. demissum* en plein soleil, nous avons vu, bien des fois, l'*imago* rester très longtemps, bien retenu en quelque sorte par le végétal, mais après de premières morsures faites rapidement et étendues, assez vite l'attaque se ralentit puis demeure faible ou très faible.

Si l'on a vu en plein champ des pousses, souvent petites, de *S. demissum* disparaitre, dévorées, c'est que les attaques vives d'insectes nouveaux arrivants s'additionnaient, les anciens perdant peu à peu leur appetit.

Des plantes caractérisées par de mauvais stationnements étaient souvent mangées avec avidité, dès qu'on empêche l'insecte de les quitter: tels apparurent dans nos essais, la variété Wolthmann du *S. tuberosum*, divers *Lycopersicum.*

La troisième phase à considérer, est l'action, avec délais, du végétal sur la physiologie de *l'imago*: aptitude à l'hivernation et surtout, comme le détaillera GRISON, taux de fécondité. Rapidement nous avons reconnu qu'il y avait là un facteur des plus marqués, réglant les taux d'attaques possibles de plantes.

Pour cette phase, le classement présentera certaines différences encore plus accentuées avec le premier classement cité, même de véritables inversions pour des plantes comme *S. demissum*, les *Petunia*, alors que pour d'autres plantes il y a parallèlisme étroit des niveaux de classement. C'est ainsi le cas, parmi les plantes très attaquées, pour *S. melongena, S. dulcamara* (surtout sous sa forme maritime naine), *S. edineuse* qui est tuberifère; parmi les plantes peu attaquées cette catégorie comprend les *Nicotiana rustica* et *tabacum*, certains *Lycopersicum.*

Physiologiquement, le Doryphore au stade *imago* parait donc très sensible à la nourriture prise, celle-ci considérée à la fois sous l'angle de la qualité et de la quantité.

L'examen de trois liens *Imago*-Plantes hôtes, montre qu'il y a, en quelque sorte, des plantes supérieurement ,,favorables'' à l'insecte parfait, tous les caractères d'affinité se présentant, pour elles, simultanément avec leur degré élevé ou le plus élevé. A un tel niveau, ce sont placés, dans nos essais, *S. edineuse, S. dulcamara* (en plein champ, non dans les haies ombragées), leurs affinités dépassant celles rencontrées chez la plupart des variétés de *S. tuberosum.*

Dans les plantes à affinités faibles ou très faibles, ce niveau bas peut résulter soit de caractères faibles partout, soit de la présence sur une seule phase d'un caractère ayant un très faible niveau, celui-ci suffisant à annuler les affinités par contre bien meilleures sur d'autres caractères.

Les affinités et immunités jouant pendant la vie larvaire, se précisent en étudiant les réactions de contact de la larve à l'égard des feuilles (tact, gustation, circulation ou immobilité, vitesse et intensité des attaques de tissus foliaires, etc....), puis les courbes de survie et les vitesses de croissance. Comme pour les *imagos,* l'aliment ingéré, par sa quantité et sa qualité, influe avec délais sur la physiologie des stades venant après la vie larvaire.

Nous avons eu d'excellentes courbes de développement sur les *Solanum marginatum, dulcamara, gilo, rostratum*; elles furent moins bonnes avec *heterodoxum, aviculare, sisymbriifolium, balbisii, N. sandere*; moins bonnes encore avec avec *demissum, commersonii, jamesii, polyadenum, nigrum, auriculatum;* le plus souvent nulles ou toujours nulles, avec les *Petunia, Salpiglossis, Hyosciamus, Datura, Nicotiana tabacum, glauca* et *rustica, S. aculeatissimum.*

Dans les deux dernières catégories, il y a parfois attaques par les jeunes larves très rapides et intéressées, puis celles-ci cessent et la mort rapide intervient le qui plus souvent (*Petunia, Salpiglossis* notamment); d'autres fois on observe des morsures très limitées ou nulles (*Datura*); enfin, dans le cas extrême, on note même l'absence de toute morsure marquée (*S. aculeatissimum*).

Les quelques exemples montrés rapidement indiquent que c'est en considérant tout un ensemble de facteurs, opérant toutefois comme un tout par leurs effets finaux, que l'on peut fixer par végétal et pour un milieu donné, une *résultante cumulative* (se jugeant seulement sur une *suite de plusieurs générations*) qui définit une sorte de „coefficient de prolifération," caractéristique, avec ses limites de variations possibles, de ce végétal.

L'étude comparative de tels coefficients est particulièrement instructive sur le plan de l'immunologie lorsqu'on l'applique à l'ensemble de la famille des Solanacées; aussi ne peut-on que souhaiter une reprise un jour prochain en détail et en tenant compte des enseignements récents obtenus sur Solanacées tuberifères, de l'étude des caractères d'immunité chez des espèces et des genres nombreux, pris parmi les Solanacées non tuberifères.

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HOST PLANT SELECTION IN THE COLORADO BEETLE LARVA

(*LEPTINOTARSA DECEMLINEATA* SAY)

(*An ethological approach to food finding in insects*)

BY

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In the food finding behaviour of the Colorado beetle larva, random searching and random biting are two apparently spontaneous behaviour elements belonging to different motor systems. Locomotion is directed by light and by host odour, biting is slightly inhibited by indifferent substrates like glass and filter paper. Initiation and maintenance of feeding are partly dependent upon different stimuli, as shown by the behaviour of the larva on *Solanum demissum* Lindl. Substances inhibiting the ingestion of food do not necessarily interfere with locomotory responses causing the insect to remain on the plant, nor do they prevent the feeding action from being released. This may lead to the cyclic process represented in fig. 6. The factors regulating intensity of motivation and threshold releasing stimulus are discussed.

In the Colorado beetle, host plant selection is primarily performed by the ovipositing female. Nevertheless, the larva is not entirely devoid of the ability to discriminate between food plants. Indeed, its abilities in food selection are such as to make the larva the preferred stage to be used in screening experiments with resistant potato hybrids.

The work of CHIN (1950) has provided a firm basis for the understanding of host finding in Leptinotarsa. It has seemed useful to discuss briefly the facts revealed by CHIN, supplemented by new ones, in the light of some ethological concepts.

A PROVISIONAL ANALYSIS OF FOOD-FINDING

The young larva, soon after hatching, consumes the remaining egg yolk. After this, it enters a period of random searching, during which it generally does not start feeding in the immediate vicinity of the former egg pod, but is apparently set in motion by some internal factor, for which we will use the ethological term motivation.

During this random searching, resulting in a more equal distribution of the larvae over the food plant, the pathway of the larva can be orientated by several environmental factors, one of which is light. The larva is equipped with two groups of six stemmata, the axes of which point in different directions (fig. 1). While the larva pursues its pathway, its head makes pendulating horizontal movements. DETHIER (1943), who observed the same movements in caterpillars, has pointed out that their function is to improve the perception of form and distance.

When the larva is placed in a gradient of light, a clear positive phototaxis is apparent.

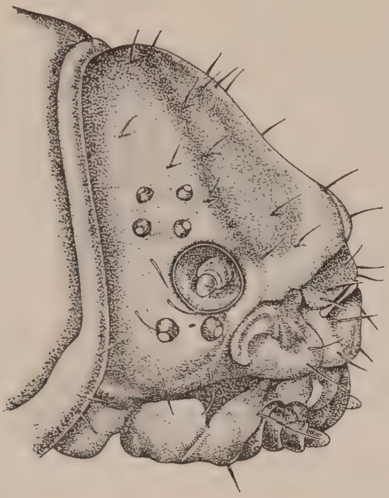

Fig. 1. Lateral view of the stemmata and position of the (retracted) antenna (DE WILDE & PET, 1957)

When a potato leaf is placed near the head of a larva removed from its foodplant, it only reacts at a close distance. Optical as well as olfactory stimuli are involved. The first is shown by the preference for green as a colour (DE WILDE & PET, 1957), which is especially clear when green is in contrast with other shades (Table I).

TABLE I

The Colorado beetle larva shows a preference for green when in competition with red, both colours being equal to the same shade of grey (DE WILDE & PET, *1957)*

COMBINATION	Gr 27—R1	Gr 18—R1	Gr 22—R1	Gr 18-Grass Grn
CHOICE	37—63	70—30	50—50	65—35
$P < 0.05$	+	+	—	+
COMBINATION	Gr 22-Grass Grn.	Grass Grn.—R1		
CHOICE	53—47	67—33		
$P < 0.05$	—	+		
COMBINATION	Yellowgrn 2-Gr 6	Gr 6-Ochre-yellow	Yellowgrn 2-Ochre-yellow	
CHOICE	52—48	57—43	62—38	
$P < 0.05$	—	—	+	

Gr 27, Gr 28, etc. = numbers of the Rietschel grey series.
R 1, Grass Grn., Yellowgrn. 2 = colours of the Hering series.
The result of 100 choices is given. The difference was tested for significance by means of binominal probability paper.

The attractiveness of the scent of solanaceous leaves is shown in the "screen test" (fig. 2) (CHIN, 1950). This simple method has the advantage that use is made of still air. Anemotactic responses need not to be taken into account. Table II demonstrates the olfactive attractiveness of the leaves of different species of Solanaceous plants.

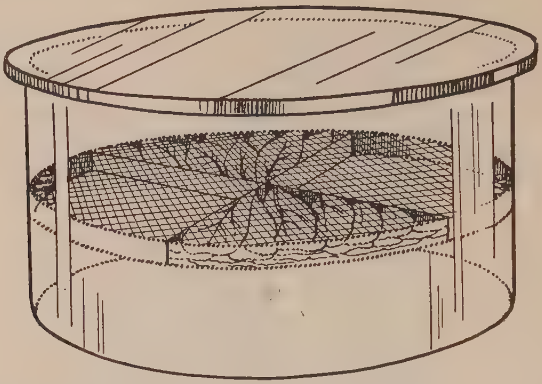

Fig. 2. Apparatus for the "screentest". The larva is placed upon a screen of wire gauze of fine mesh, to avoid optical or tactile contact with the leaves placed in the lower compartment (CHIN, 1950)

TABLE II

Preference for host plant odour by the Colorado beetle larva, as demonstrated by the screen test (CHIN, *1950) (% of positive choices with mean error, 50% being neutral)*

Solanum tuberosum	58,2 ± 2,1
S. demissum	59,2 ± 2,5
Petunia hybrida	57,2 ± 2,4
Doronicum sp.	49,2 ± 2,2
S. tuberosum (antennae amputated)	51,0 ± 2,5
S. tuberosum (antennae + palpae amputated)	48,8 ± 2,6

When the larva is removed from the plant, both factors may now be responsible for host finding. When it is on the food plant, they result in a tendency to stick to it, and move to places with highest illumination.

Next to "random searching", there is another type of activity which can occur without the interference of specific substrata, though it may happen more readily in the presence of such. This is „random biting"[1]. The searching larva bites a variety of substances. When a new substratum is offered to the larva, the time elapsing before biting occurs may be used as a measure of its attractiveness. We then see, that leaves of very different plants as *Solanum, Doronicum* and *Pelargonium* are bitten more readily than neutral substrates like glass or filter paper. Remarkable enough, removal of the palpae and antennae enhances the biting responses towards neutral substrates, suggesting an inhibitory influence of the lastnamed. We will call this inhibitory factor the "palp factor" (Table III).

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[1] "random" is used here in the sense of "whereever the larva walks". It occurs only in the underlying substrate and is not "random" in this sense.

TABLE III

Enhancement of biting in the underlying substrate brought about by the amputation of palpae and antennae (CHIN, *1950)*

SUBSTRATA	NORMAL LARVA					OPERATED LARVA				
	1	2	3	4	5	1	2	3	4	5
GLASS PETRI DISH COVER	—	—	—	+	--	+	+	-	+	—
DRY FILTER PAPER	—	—	—	—	—	+ +	+++	—	+ +	+
WETTED FILTER PAPER	+	—	—	+	—	+ +	+++	+++	+ +	+++
DORONICUM LEAF	+	+	+	—	—	+++	+++	+ +	+	+++
GERANIUM LEAF	+ +	+++	+++	+ +	+++	+++	+++	+++	+ +	+++
PETUNIA LEAF	+++	+ +	+++	+ +	+++	+++	+++	+++	+++	+++
DEMISSUM LEAF	+++	+	+ +	+	+++	+ +	+++	+++	+ +	+++
TUBEROSUM LEAF	+++	+++	+++	+	+++	+++	+++	+++	+++	+++

—	30 minutes elapsed without any biting action
+	biting action more than two minutes after the larva was introduced on the substratum
++	biting action between one and two minutes after the larva was introduced on the substratum
+++	biting action less than one minute after the larva was introduced on the substratum

As soon as the larva bites a leaf belonging to its host plant series, a new type of action is released. We may now observe the larva "shaving" a certain part of the surface, if a dense pilosity is present, as in some lines of *Solanum demissum.* The larva subsequently perforates the leaf epidermis. The beginning of the feeding action is marked by the withdrawal of the antennae into the head capsula (fig. 3). During searching, these telescope-like appendages are held in a stretched position, thus enabling the sensilla basiconica on the terminal segment to fulfill their function.

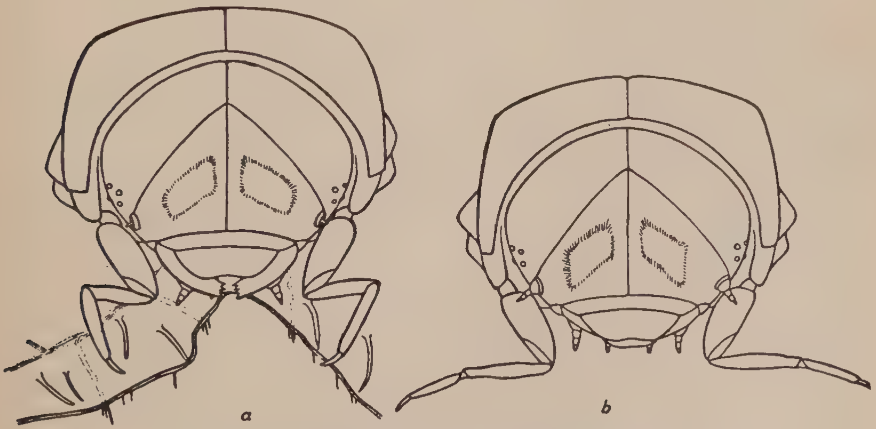

Fig. 3. Colorado beetle larva showing antenna
a. during feeding; b. when searching (CHIN, 1950)

After the feeding action has started, the behaviour of the larva is still greatly influenced by the internal condition of the leaf, as is shown when different *Solanum* species are compared (fig. 4).

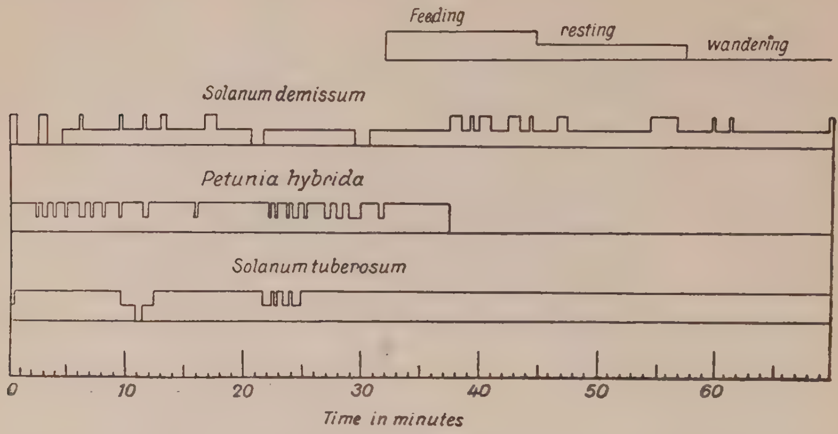

Fig. 4. The activities of the fourth instar larva on the leaves of Solanum tuberosum and Solanum demissum (CHIN, 1950)

On potato leaves we observe continuous feeding, interrupted by short periods of rest. On resistant lines of *Solanum demissum,* most of the time is spent resting and wandering, interrupted by short feeding periods. This results in quite different feeding patterns (fig. 5).

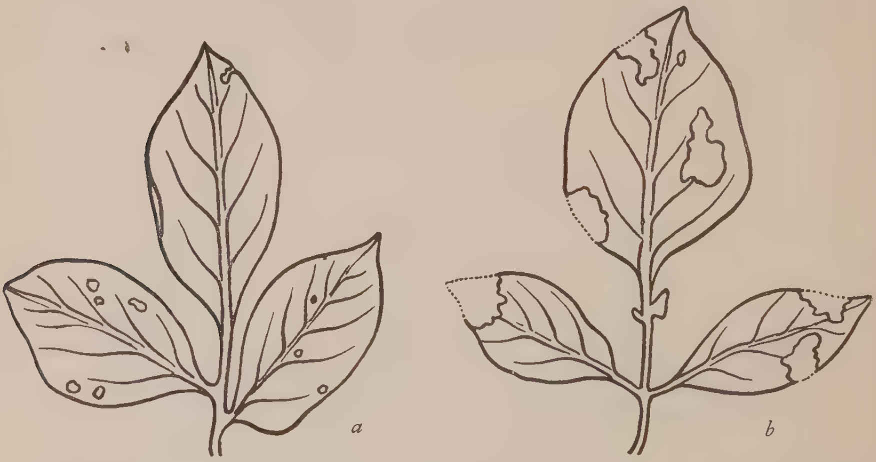

Fig. 5. Feeding pattern of the third instar larva a. on S. demissum; b. on S. tuberosum

Apparently the sense organs on the mouth parts are involved, for removal of the palpae results in enhanced feeding on plants rejected before. Furthermore, we may produce the same pattern as obtained on *Solanum demissum* by infiltration of potato leaves with one per cent acetic acid.

Putting the above-named observations into a scheme, we find that host selection is chiefly made on a chemosensory basis (fig. 6).

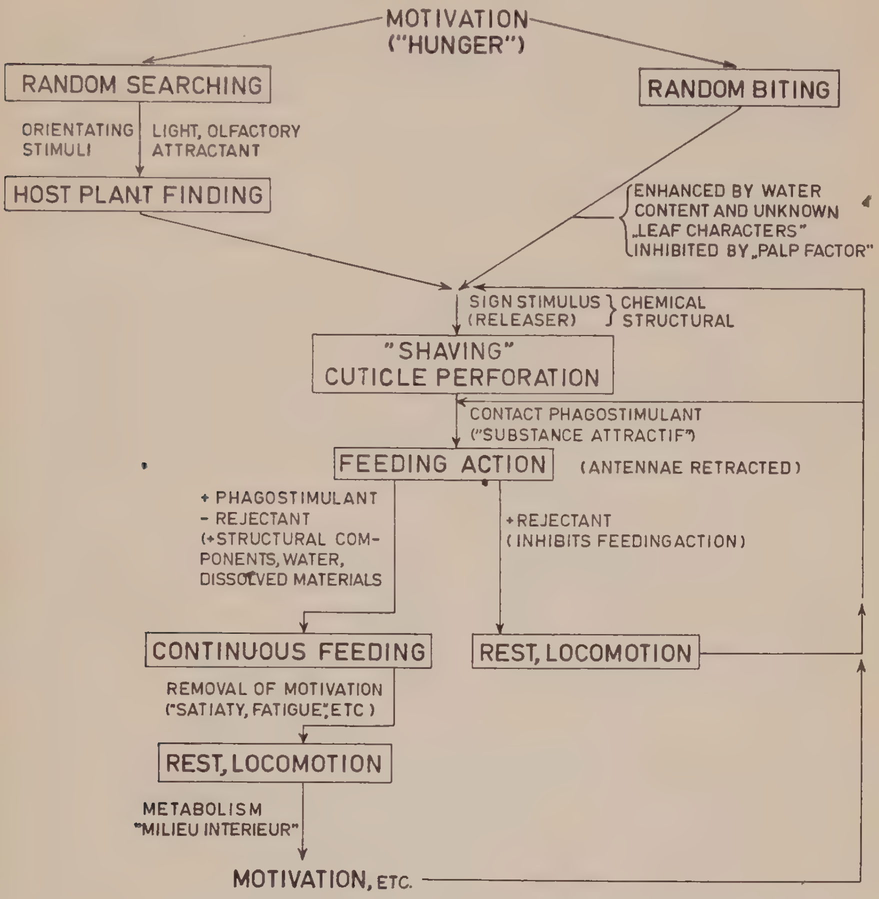
MOTIVATION ("HUNGER")
RANDOM SEARCHING
RANDOM BITING
ORIENTATING STIMULI
LIGHT, OLFACTORY ATTRACTANT
HOST PLANT FINDING
ENHANCED BY WATER CONTENT AND UNKNOWN „LEAF CHARACTERS"
INHIBITED BY „PALP FACTOR"
SIGN STIMULUS (RELEASER)
CHEMICAL
STRUCTURAL
"SHAVING" CUTICLE PERFORATION
CONTACT PHAGOSTIMULANT ("SUBSTANCE ATTRACTIF")
FEEDING ACTION
(ANTENNAE RETRACTED)
+ PHAGOSTIMULANT
- REJECTANT
(+STRUCTURAL COMPONENTS, WATER, DISSOLVED MATERIALS
+REJECTANT (INHIBITS FEEDING ACTION)
CONTINUOUS FEEDING
REST, LOCOMOTION
REMOVAL OF MOTIVATION ("SATIATY, FATIGUE", ETC)
REST, LOCOMOTION
METABOLISM "MILIEU INTERIEUR"
MOTIVATION, ETC.

Fig. 6. Scheme of the sequence of activities leading to host finding and acceptance or rejection by the colorado beetle larva

PHAGOSTIMULANTS AND REJECTANTS

As regards the substances involved, much has still to be discovered. The olfactory attractive substance is unknown. It is certainly not identical with the "substance attractif" as mentioned by RAUCOURT & TROUVELOT (1936). This last-named substance is very probably a releaser of the feeding action [1]). It has been purified to some extent by CHAUVIN (1952), who defines it as a hygroscopic flavoglucoside. Much more is known of the chemical nature of the "resistance-factors" present in *Solanum demissum*, *Solanum chacoense* Bitter, the tomato and some other Solanaceae. It appears that, contrary to the statements of HESSE & MEIER (1950) the attractive principles present in these species are much the same as in the potato. However, while the alkaloid solanine in the concentration present in *Solanum tuberosum* L. apparently does not provide a chemical stimulus to the beetle, the alkaloids demissine, tomatine, and leptine are strongly rejected in the concentration present in the plant (KUHN et al. 1947, 1950, 1957). Their presence adds a repellent (perhaps we might better use the term rejectant, as proposed by THORSTEINSON) to the otherwise acceptable food.

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[1]) The term "phagostimulant", as proposed by THORSTEINSON (1957) has been adopted by us, though to our opinion the term "releasing substance" would fit better. Similarly, BAERENDS (1950), discussing the behaviour of flower-visiting insects, states that "........to some insects odour................ means a releasing stimulus."

DISCUSSION

In our attempt at an analysis of host finding and recognition in this oligophagous beetle, we have been primarily interested in the type of information which the insect obtains from its food. This information can only be obtained through the sense organs. These in their turn select from the "Umwelt" the stimuli which, separately or in combination release or direct locomotory or feeding responses.

It seems of importance to stress once more the difference between the properties of plant substances as nutritional elements and as factors in host finding and initiation and maintenance of feeding. In dealing with the last category, DETHIER (1953) distinguishes between 1. orientation, 2. biting responses and 3. maintenance of feeding. In addition we would like to stress the role of plant substances as releasers and inhibitors of the feeding action. We also find that, in the colorado beetle larva, initial biting is probably an innate activity requiring internal motivation.

What may be the role of the nutrients in this complicated behaviour pattern ?

To KENNEDY & BOOTH (1951, p. 60), stating "...... one might expect aphids to have evolved some tastes for things that are good for them, in an immediate nutritional sense, and other tastes for things that are less or not at all good for them, but are valuable instruments of ecological specialization", one may object that even man cannot taste proteins or lipoids, nor the many vitamins that are indispensable parts of his nutrition.

Though the statement by FRAENKEL (1953, p. 99): "......That it is the presence of odd chemical substances in which plants differ, such as glucosides...... which renders the plant a suitable or unsuitable food" is, according to the same author"...... an admittedly extreme and onesided view", it has the value that a sharp distinction is made here between the behaviour components of food selection and nutrition as such.

As found by THORPE c.s. (1947), the movements of polyphagous wireworms like *Agriotes lineatus* L. and *A. obscurus* L. are orientated, and their feeding action released, by substances generally occurring in plants like glucose and asparagine. Oligo- or monophagous insects require for their food intake much more specific chemical substances which, in the relation plant-insect have an orientating and releasing function.

The necessity of such "odd substances" does of course not exclude that components of a more general occurrence, used as nutrients by the insect, may also act along the sensory pathway and play a role in food selection. There are numerous observations to prove the contrary (see THORSTEINSON, 1958). It may even represent the more primitive situation, the specialized feeding habits of oligophagous insects being due, as it were, to the formation of a conditioned reflex on the "odd substance", which in the course of their evolution has become part of their inherited behaviour pattern.

Once ingested, the nutrients most probably intervene by either deminishing the intensity of motivation ("hunger condition"), or increasing the threshold for the releasing stimuli of the feeding action. Recent experiments by DETHIER throw some light on the general aspects of this problem.

An accurate analysis of the factors controlling the ingestion of carbohydrates by the blowfly, *Phormia regina* Meig., has led DETHIER to the conclusions, that (a) there is no relation between the amount of sugar taken in and its nutritional value (DETHIER, 1956) and (b) raising the level of glucose in the haemolymph by means of injection does not influence the threshold concentration of glucose with regard to the sucking response (DETHIER, 1957). This would mean that in the blowfly, neither the feeding motivation nor the liminal releasing stimulus for the feeding action are regulated by the nutritional substance via the haemolymph.

We have no data to confirm or to reject either of these points in phytophagous insects.

RÉSUMÉ

1. Rapidement après l'éclosion, le premier stade larvaire de *Leptinotarsa* fait au hasard des mouvements d'exploration.
2. Ces mouvements peuvent être dirigés par la lumière (intensité, couleur) et par l'odeur de la feuille de *Solanum*.
3. Chez une larve en exploration, la morsure faite au hasard est un autre élément du comportement spontané, qui peut être inhibé par les impulsions reçues par les palpes.
4. Le début et le maintien de l'alimentation dépendent partiellement de différents stimuli, comme le montre le comportement de la larve sur *Solanum demissum*. Les substances inhibant l'ingestion d'aliment n'empêchent pas nécessairement l'acte alimentaire d'être déclanché.
5. Ceci peut aboutir à un cycle fermé représenté dans le diagramme.
6. Le rôle de constituants nutritifs dans la sélection de la plante-hôte est discuté.

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DISCUSSION

B. TROUVELOT asked whether the reaction to the stimuli mentioned occurred periodically, in relation to the rhythm of illumination. The answer was, that this is indeed the case; it has been proved that the beetle as a whole shows a diurnal rhythm of activity. It must be concluded that the feeding motivation shows a similar rhythm.

J. S. KENNEDY wondered whether the "palp factor" would consist of tactile stimuli provided by hard or brittle substances like glass or paper. He further asked whether prolonged stimulation of this kind would result in a lack of "hungry" behaviour shown by the starved larva. The author is indeed inclined to think that the inhibitory "palp factor" results from tactile stimuli unbalanced by host odour or taste. The fact that a palpectomised larva resembles in its behaviour a starved larva means that in the latter case the threshold for the palp factor is probably increased.

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THE CHEMOTACTIC INFLUENCE OF PLANT CONSTITUENTS ON FEEDING BY PHYTOPHAGOUS INSECTS

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The available evidence is in agreement with the postulate that all phytophagous insects require chemotactic (usually gustatory) stimuli to release feeding activity. Some of the soluble nutrients in plants serve this function. In the case of oligophagous insects food selection is further guided by perception of non-nutritive substances (token stimuli) of limited botanical distribution. The food range of all phytophagous insects is circumscribed by the distribution of repellants, rejectants and toxins. These concepts are developed and outlined in symbolic form.

Food plant selection or botanical specificity is brought about through two phases of insect behaviour. *viz* (1) food finding and (2) food acceptance. The odourous constituents of plants may assist insects in finding their food plants but little is known about the identity of chemicals that serve this function nor about the precise behaviour mechanisms displayed by insects in response to such stimuli.

Food acceptance is manifest either by the act of oviposition or by feeding. Only the second of these two types of response will be considered in this discussion. The term "acceptance" is preferable to "selection" since it is applicable whether or not a choice is available and does not imply any psychological behaviour on a higher level. As the ethological aspects of feeding by phytophagous insects have already been discussed in this symposium in a highly satisfactory manner by Professor DE WILDE, it will be appropriate to focus attention on the chemical plant constituents and the nature of their influence on feeding responses.

In the past, more notice has been taken of the token feeding stimuli provided by the non-nutritional constituents of plants such as the glycosides. The rather striking responses that can be evoked by token stimuli are illustrated in Table I. Similar data have been given previously (THORSTEINSON, 1953). It is evident that the diamond-back moth larva does not accept a mixture of nutrients unless it contains at least a small amount of a specific token phagostimulant (mustard oil glucoside) which occurs in its natural food plants.

However, it would be rather rash to generalize from the behaviour of one or even several insect species and, *a priori*, it should seem strange that nutrient plant constituents do not affect the palatability of food to many insects. Indeed, one of the universally present substances in plants, namely sucrose, has long been known to be avidly acceptable to numerous phytophagous and other insects species (BECK, 1956). Although several investigations have studied the responses of insects to sugars, quinine, salts and acids the question as to whether insects perceive the taste of important nutrient constituents of their food such as amino acids, amides, vitamines etc. has been largely neglected. An important exception is the work of THORPE ET AL (1947) on the food finding of wireworms.

TABLE I

Feeding Responses to a token phagostimulant (sinigrin) by Plutella maculipennis Curt. as Measured by Frass Pellet Count

DIET	MEAN FRASS COUNT (5 Replicates)	STANDARD ERROR
1. Nutrient Agar*	117.8	15.8
2. Nutrient Agar + 0.1% Sinigrin	306.4	16.1
3. Black mustard leaf powder (2%) Agar	311.8	21.4

* Nutrients comprise glucose 6%, soluble casein 2%, triolein 0.5%, yeast 2%.

Of course, the physical obstacles confronting wireworms in their search for food would seem to demand some special compensation in the form of chemotactic guidance and THORPE ET AL have demonstrated elegantly that nature has provided them accordingly. It is not improbable however that leaf-eating forms also have a compelling need to appraise the quality of food such as would be provided by a perception of soluble constituents. As the event has proved, it has been worth looking for such effects. Indeed, the number and variety of substances that stimulate feeding by insects has exceeded our expectations.

By the use of "artificial leaves" prepared from thin discs of elder pith (a technique first introduced by the president of this Symposium, Professor TROUVELOT) it is possible to obtain satisfactory estimates of the phagostimulant activity for chewing insects of any soluble substance that may be of interest. By the use of this method including the refinements introduced by CHAUVIN (1952) we have studied the feeding response of several phytophagous insects including oligophagous and polyphagous forms (especially grasshoppers). An outline of the salient findings follow:

As we expected, polyphytophagous insects are stimulated to feed by sucrose which is a more active phagostimulant than glucose. For grasshoppers the optimum concentration is about 0.02 M under the conditions of our tests. An unexpected finding was that one oligophagous insect, *Leptinotarsa decemlineata* Say, is stimulated to feed by sucrose even when no token phagostimulant is present and, as for the grasshoppers, 0.02 M is the approximate optimal concentration but in this case little feeding response is obtained below this concentration.

As was reported previously (THORSTEINSON, 1956), a number of metabolic constituents of plants possess phagostimulant activity for various phytophagous insects. These substances include amino acids, amides and vitamines such as thiamine and ascorbic acid.

In view of these results and the recent finding by R. H. DADD (1957) that ascorbic acid may constitute a vitamine requirement for locusts one is tempted to speculate that the gustatory sense of insects may assist them in "selecting" food of suitable nutritional quality. Of course it is too improbable that all the varied nutritional components could be appraised by insects in this way. However, it is not entirely implausible that some of the nutrient substances perceived by the gustatory sense of insects may serve as indicators of nutritional quality. If so, such substances, although of nutritional consequence, would nevertheless serve as ,,token" phagostimulants. This suggests that the distinction between token and nutritional feeding stimulants is not distinct. At any rate, it seems clear that there is a considerable correlation between the chemotactic stimuli that govern feeding and the nutritional requirements of insects. This conclusion is sufficiently probable *a priori* but there has been a reluctance to accept it (Lipke and Fraenkel, 1956) pending actual experimental evidence. This evidence appears now to be forthcoming.

In view of the fact that some chemical constituents of plants function both as nutrients and as phagostimulants for insects it is of interest to consider what other dual effects may be manifested by plant constituents. Table II suggests some possibilities:

Table II

Dual Effects of Chemical Plant Constituents on Feeding and Metabolism of Insects

	Feeding Stimulant or Acceptant *	Nutrient	Rejectant *
Feeding Stimulant or Acceptant *	T		
Nutrient	Ns		
Rejectant *	—	?	
Toxin	?	?	+

* The terms "acceptant" and "rejectant" have been adopted in place of "attractant" and "repellent" where the responses of the insects do not involve locomotion towards or away from the substrate, following discussions with R. H. Dadd and D. Goodhue, Imperial College, London.

A substance that stimulates feeding but has no other effect is a token phagostimulant (T). If however, it is also a nutrient (for example, sucrose) we may call it a sapid nutrient (Ns) to distinguish it from nutrients that are vapid or tasteless and do not affect feeding responses. A feeding stimulant cannot be also a repellent since this is a contradiction in terms. A substance that is both a feeding stimulant and a toxin is an interesting possibility since it should have valuable applications as an insecticide but we do not know of such a substance. A repellent nutrient seems somewhat unlikely, yet Nuorteva (1952) has demonstrated that leafhoppers avoid sap of high sugar concentration. A toxic nutrient would seem to be a contradiction in terms unless we recall that any substance is likely to be toxic in sufficiently high dosage. Toxic effects of sugars have been reported by Fraenkel (1955). Finally, a toxic repellent is not an unlikely combination and demissin is probably an example of such a substance for *Leptinotarsa.*

In order to summarize the conditions required for normal feeding and development by phytophagous insects, the following symbolic scheme is presented.

$F \underset{\leftarrow}{\longrightarrow} I$

Normal feeding (F) implies ingestibility (I) but I is not sufficient to ensure normal feeding.
Similarly,

$F \underset{\leftarrow}{\longrightarrow} P_T$ (token stimulus factor in acceptability)

$F \underset{\leftarrow}{\longrightarrow} P_{N_s}$ (sapid nutrient factor in palatability)

$F \underset{\leftarrow}{\longrightarrow} pH$ (optimum pH)

$F \underset{\leftarrow}{\longrightarrow} -R\ -P$ (absence of rejectants and poisons)

$F \underset{\longleftarrow}{\longrightarrow} I + P_T + P_{N_s} + pH - R - P$

Normal feeding implies all the factors on the right which *together* are sufficient to induce normal feeding.
Furthermore,

$D \underset{\leftarrow}{\longrightarrow} F$ (Normal development implies normal feeding but normal feeding is not sufficient for normal development).

Similary,

$D \underset{\leftarrow}{\longrightarrow} N_c$ (complete complement of nutrients)

$N_c \underset{\longleftarrow}{\longrightarrow} N_s + N_v$ (complete complement of nutrients implies both sapid and vapid nutrients)

$D \underset{\longleftarrow}{\longrightarrow} F + N_c$ (normal development implies normal feeding and a complete complement of nutrients)

But N_s is common to both F and N_c
Hence,

$D \underset{\longleftarrow}{\longrightarrow} F + N_v$ (normal development implies normal feeding on a diet that includes both sapid and vapid nutrients)

In an earlier paper (THORSTEINSON, 1953), I pointed out that we should normally expect all insects to require some gustatory stimulus to initiate and sustain feeding activity. If such stimuli for a given insect species include token stimulants with a restricted botanical distribution, the insect will be oligophagous or monophagous. The phagostimulants required by polyphagous insects must necessarily have a wide botanical distribution. In the latter group, host plant acceptance will be governed by the botanical distribution of toxic or rejectant substances that mask or interfere with the effect of the phagostimulants but this should not obscure the significance of the phagostimulants to an understanding of the problem of host plant relationships of insects.

RÉSUMÉ

L'importance des stimulants alimentaires "token"1) a été clairement démontrée par exemple chez *Plutella maculipennis* Curt., *Pieris brassicae* L. et *P. rapae* L., lesquels, à l'état larvaire, reconnaissent leur plante-hôte, les Crucifères, au goût de glucosides de l'huile de moutarde tels que la sinigrine et la sinalbine. Bien qu'elles soient presque les seules démonstrations indiscutables de l'action de ces stimulants "token" parmi les insectes phytophages il a été admis que ce phénomène explique en général la sélection de la plante hôte par les insects

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1) "Token" = Stimulus favorisant la reconnaissance de la nourriture, mais qui lui-même n'a pas de valeur nutritif.

oligophages et que la présence de substances désagréables au goût dans les végétaux est suffisant pour expliquer les préférences de plantes alimentaires des insectes polyphages. Cependant, ces vues ne rendent pas compte du fait que ces „phagostimulants "token" peuvent exiger la présence de constituants nutritifs afin d'obtenir une réponse alimentaire chez les insectes oligophages. En outre les insectes polyphages demandent des stimuli chimiotactiques positifs pour déclancher une activité alimentaire. Aucun effet inhibiteur de substances végétales désagréables n'est simplement superposé à ce phénomène de base.

Un autre fait significatif est que le saccharose, constituant universellement distribué chez les végétaux, est avidement goûté par de nombreux insectes, et on pourrait penser *à priori* que son rôle est important dans la nature comme stimulus effecteur d'alimentation chez les insectes phytophages. De récentes investigations ont montré que d'autres substances nutritives variées telles que le glucose, des acides aminés, des amides et des vitamines peuvent aussi déclancher d'une manière indépendante l'activité alimentaire.

Une interprétation totale de la connaissance utile à présent est que de nombreuses conditions sont exigées pour rendre compte des phénomènes d'alimentation chez les insectes et de la sélection de l'hôte. Ceci inclut la présence de stimulants alimentaires "token" (si nécessaires), la présence de certains constituants sapides et l'absence de substances répulsives ou désagréables au goût. Tandis que ces conditions, admettant un substrat ingestible, sont nécessaires et suffisantes pour une alimentation normale un développement satisfaisant exige en plus la présence de constituants nutritifs essentiels autres que les substances sapides déjà mentionnées et l'absence de toxines.

Certains constituants végétaux peuvent avoir plusieurs fonctions dans ce schéma et quelques exemples sont considérés.

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ÜBER EINIGE INHALTSSTOFFE DER SOLANACEEN UND IHRE BEDEUTUNG FÜR DIE KARTOFFELKÄFERRESISTENZ [1])

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Unsere heutigen Kenntnisse über die natürlichen pflanzlichen Resistenzstoffe gegen den Kartoffelkäfer (*Leptinotarsa decemlineata* Say), insbesondere auf dem Gebiet der *Solanum*-Alkaloide, werden zusammengestellt. Gleichzeitig wird versucht, den Wirkungsmechanismus dieser Stoffe zu deuten.

Chemische Faktoren werden in zunehmendem Maße zur Deutung von Resistenzerscheinungen herangezogen. Eine auf die chemische Zusammensetzung der Pflanzen zurückführbare Widerstandsfähigkeit gegenüber Schädlingen kann folgende Ursachen haben:

1. Ausreichendes Vorkommen von entwicklungshemmenden sowie toxisch oder „futtervergällend" wirkenden Inhaltsstoffen,
2. unzureichender Nahrungswert der Wirtspflanze und
3. das Fehlen wirksamer „Lock-" und „Fraßstoffe".

Der Kartoffelkäfer und seine Larven sind im Rahmen der Pflanzenfamilie der Solanaceen oligophag. Durch das Zusammenspiel der genannten Faktoren tritt bei den verschiedenen Gattungen, Arten, Formen und Herkünften eine graduell sehr unterschiedliche Kartoffelkäferresistenz in Erscheinung.

Über das Nahrungsbedürfnis phytophager Insekten im allgemeinen und des Kartoffelkäfers im besonderen sind unsere derzeitigen Kenntnisse noch sehr unzureichend, vor allem da die Untersuchung der Frage, welche Bedeutung speziellen Nährstoffgruppen oder gar bestimmten, chemisch definierbaren Nahrungsbestandteilen zukommt, ausserordentlich schwierig ist. Ebenso offen ist das Problem der Fraß- und Lockstoffe, obgleich gerade hier in letzter Zeit, insbesondere in physiologischer Richtung, beachtliche Fortschritte erzielt wurden.

Einfacher dagegen ist die chemische Bearbeitung der zusätzlich in widerstandsfähigen Pflanzen vorkommenden, resistenzbedingenden Inhaltsstoffe, da geeignete Testmethoden auch bei phytophagen Insekten möglich und bekannt sind.

Als von KUHN und Mitarbeitern (KUHN und LÖW, 1947; KUHN und GAUHE, 1947; KUHN, LÖW und GAUHE, 1950) vor einigen Jahren mitgeteilt worden war, dass die Kartoffelkäfer-Larvenresistenz der mexikanischen Wildkartoffel *Solanum demissum* sowie einiger *Lycopersicon*-Arten auf die in diesen Pflanzen vorkommenden Alkaloidglykoside Demissin (VII) bezw. Tomatin (XII) zurückzuführen ist, wurde den chemischen Resistenzstoffen im allgemeinen und den Alkaloiden im besonderen ein erneutes starkes Interesse entgegengebracht.

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[1]) Vgl. K. SCHREIBER: Natürliche pflanzliche Resistenzstoffe gegen den Kartoffelkäfer und ihr möglicher Wirkungsmechanismus, *Der Züchter* 27, 289—299 (1957).

Im Gegensatz zu Demissin (VII) und Tomatin (XII) üben das α-Solanin (II) und α-Chaconin (III) unserer Kulturkartoffel (*Solanum tuberosum*) diese Schutzwirkung nicht aus (KUHN und GAUHE, 1947; KUHN und LÖW, 1955; STEPANOWA und MIRONOWA, 1955; SCHREIBER, 1955; BUHR, TOBALL und SCHREIBER, 1957). Eine gleiche Unwirksamkeit muss den in einigen nicht knollentragenden *Solanum*-Arten (*Solanum sodomeum, Solanum aviculare, Solanum auriculatum, Solanum nigrum*[1]) u.a.) vorkommenden Alkaloiden Solasonin (IX) und Solamargin (X) zugesprochen werden (BUHR, TOBALL und SCHREIBER, 1957).

Demissin und Tomatin führen nicht zu einer akuten Vergiftung der Larven, sondern sie wirken als Abschreckstoffe, die das Futter für das Insekt „vergällen" und es so dem Hungertode ausliefern. Darüber hinaus wurden jedoch bei gelegentlich überlebenden Tieren, vor allem an nicht vollresistenten Pflanzen, die nur einen geringen Demissin- bzw. Tomatingehalt aufwiesen, Entwicklungsstörungen beobachtet (TROUVELOT und GRISON, 1935; CHIN, 1950).

Bei der Betrachtung der Strukturformeln (I—XIII) fällt auf, dass die resistenzbedingenden Alkaloide Demissin und Tomatin im Gegensatz zu den unwirksamen Verbindungen eine aus vier Monosacchariden (1 Mol. D-Galaktose, 2 Mol. D-Glukose und 1 Mol. D-Xylose) aufgebaute Kohlenhydratkomponente besitzen, also Tetraoside darstellen. In Demissin und Tomatin kommt somit die Pentose D-Xylose vor, in den Triosiden α-Solanin, α-Chaconin, Solasonin und Solamargin dagegen neben D-Galaktose und D-Glukose die Methylpentose L-Rhamnose. Außerdem ist die in den unwirksamen Alkaloiden auftretende $\triangle^5$-Doppelbindung in den beiden wirksamen Verbindungen nicht vorhanden.

Ein uns zur Verfügung stehendes, sehr umfangreiches Sortiment an *Solanum*-Arten bot Gelegenheit, die oben aufgeworfene Frage nach den möglichen Beziehungen zwischen der chemischen Konstitution der *Solanum*-Alkaloide und ihrer Kartoffelkäferwirksamkeit durch die chemische Untersuchung weiterer, insbesondere resistenter Arten, zu überprüfen.

So konnten wir zum Beispiel aus dem sehr heterozygot resistenten *Solanum polyadenium* 2 Alkaloidglykoside isolieren, die in der Zusammensetzung ihrer Kohlenhydratkomponenten den bereits genannten Alkaloidpaaren α-Solanin-α-Chaconin (II und III) bzw. Solasonin-Solamargin (IX und X) entsprechen; allerdings enthalten diese *polyadenium*-Glykoalkaloide keine L-Rhamnose, sondern D-Xylose.

Während das resistenzbedingende Tetraosid, das nach unseren Befunden nur gelegentlich in den Pflanzen vorkommt, aus dem Aglykon Tomatidin (XI), 1 Mol. D-Galaktose, 2 Mol. D-Glukose sowie 1 Mol. D-Xylose aufgebaut und mit dem Tomatin (XII) der *Lycopersicon*-Arten identisch ist, besitzt das Triosid, über das wir bereits vor einiger Zeit kurz berichteten (SCHREIBER, 1954 a; BUHR, 1955), nur eine geringe Larvenwirksamkeit (BUHR, TOBALL und SCHREIBER, 1957).

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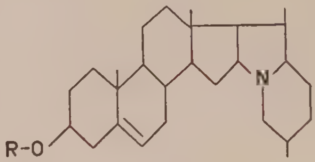
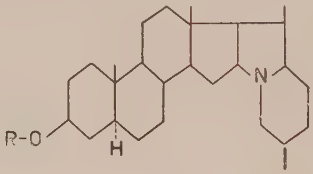
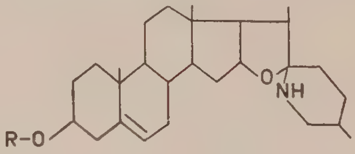
[1]) In zahlreichen Varietäten und Herkünften von *Solanum nigrum* und verwandten Arten, die im allgemeinen eine gute Kartoffelkäferresistenz zeigen, konnten wir papierchromatographisch insgesamt 6 Alkaloidglykoside nachweisen, die wir vorläufig mit alpha-, bèta-, gamma-, delta-, epsilon- und zèta-Solanigrin bezeichnen möchten. Das Aglykon dieser Glykoalkaloide, die sich von den *tuberosum*-Alkaloiden eindeutig unterscheiden, ist mit Solasodin identisch. Delta- und gamma-Solanigrin, die vermutlich Hauptalkaloide darstellen, konnten nach präparativer Gewinnung als Solasonin und Solamargin identifiziert werden (SCHREIBER, 1957 b).



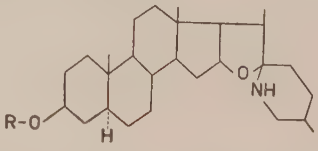
I R = H . . . . : Solanidin
II R = L-Rhamnosyl-D-glukosyl-D-galaktosyl . . . : α-Solanin
III R = L-Rhamnosyl-L-rhamnosyl-D-glukosyl . . . : α-Chaconin
IV R = D-Xylosyl-D-xylosyl-D-glukosyl . . . . : Solacaulin
V R = D-Xylosyl-D-glukosyl-D-glukosyl-D-galaktosyl : Tetrosid aus der Ser. *Acaulia.*



VI R = H . . . . : Demissidin
VII R = D-Xylosyl-D-glukosyl-D-glukosyl-D-galaktosyl : Demissin



VIII R = H . . . . : Solasodin
IX R = L-Rhamnosyl-D-glukosyl-D-galaktosyl . . . : Solasonin
X R = L-Rhamnosyl-L-rhamnosyl-D-glukosyl . . . : Solamargin

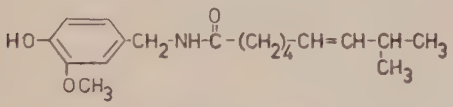


XI R = H . . . . : Tomatidin
XII R = D-Xylosyl-D-glukosyl-D-glukosyl-D-galaktosyl : Tomatin
XIII R = D-Xylosyl-D-xylosyl-D-glukosyl . . . . : Polyadenin

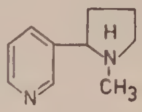
Dieses *polyadenium*-Triosid, für das wir die Bezeichnung Polyadenin (XIII) vorschlagen, ist bisher in der Natur noch nicht aufgefunden worden. Es ist aus Tomatidin (XI) und den Kohlenhydraten D-Glukose (1 Mol.) und D-Xylose (2 Mol.) zusammengesetzt.

Ein ähnliches xylosehaltiges Alkaloidpaar isolierten wir aus der Wildkartoffelserie der *Acaulia.* Neben Solacaulin (IV) (SCHREIBER, 1954 b; SCHREIBER, 1956) kommt hier in geringen Mengen, vor allem in den Wildkartoffelarten *Solanum* *schreiteri* und *Solanum punae*, ein zusätzlich D-Galaktose enthaltendes Tetraosid (V), dem ebenfalls eine Larvenwirksamkeit zugesprochen werden muss.

Während es nach unseren Mühlhäuser Untersuchungen gesichert erscheint, dass für die Resistenz der *Capsicum*-Arten das auch in den Blättern dieser Pflanze vorkommende Säureamid Capsaicin (XIV) und für die Widerstandsfähigkeit der Gattung *Nicotiana* das hier stark verbreitete und für die Kartoffelkäferlarven überaus toxische Alkaloid Nicotin (XV) verantwortlich zu machen sind, führten die an zahlreichen Instituten durchgeführten Arbeiten zur Auffindung und Identifizierung der resistenzbedingenden Stoffe der Wildkartoffelserie der *Commersoniana/Glabrescentia* bisher nur zu Teilergebnissen.



XIV Capsaicin



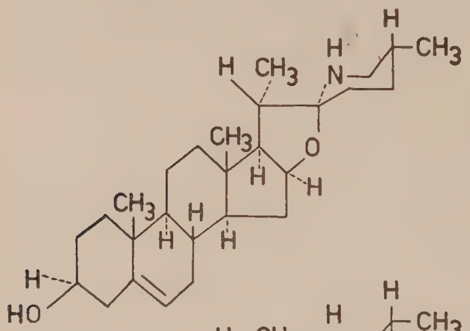
XV Nicotin

Von uns konnten aus einem homozygot larven- und käferresistenten *Solanum parodii* zwei vermutlich unterschiedliche resistenzbedingende Stoffe angereichert werden (durch Diäthyläther- bzw. Methanol-Extraktion der getrockneten Blätter). Ob einer dieser Resistenzfaktoren mit den von Kuhn und Löw (1957) kürzlich in *Solanum chacoense* aufgefundenen „Leptinen" identisch ist, konnte noch nicht geklärt werden. Die Leptine sind sehr gut wasserlösliche, durch Ammoniak nicht fällbare, zwitterionähnliche Alkaloidglykoside, die nach Abspaltung einer sauren Komponente in sogenannte „Leptinine" übergehen. An dem Aufbau dieser Glykoside sollen neben dem Aglykon „Leptinidin" (vermutlich ein Dihydroxyalkamin) D-Galaktose, D-Glukose und L-Rhamnose beteiligt sein.

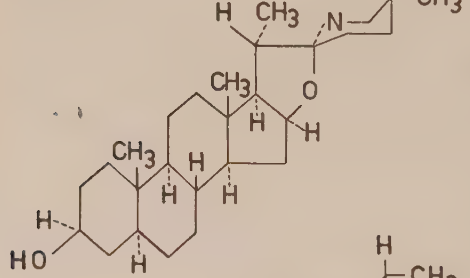
Ein durch Ammoniak ebenfalls kaum fällbares Alkaloidglykosidgemisch konnten wir aus einer kleinblättrigen Herkunft von *Solanum dulcamara* isolieren. Papierchromatographisch wurden in dem Gemisch 3 Alkaloide nachgewiesen, die wir mit α-, β- und γ-Soladulcin bezeichnen (Schreiber, 1957 c). Das Aglykon dieser Glykoside ist identisch mit 5α-Solasodanal-(3β) (Dihydro-solasodin, XVII), das mit Tomatidin (5 α-Tomatidanol-(3β), XVIII) stereoisomer und aus Solasodin ($\triangle^5$-Solasodenol-(3β), XVI) durch katalytische Hydrierung darstellbar ist (Schreiber, 1957 a; Schreiber, 1957 c; Briggs, Newbold und Stace, 1942; Briggs, Harvey, Locker, McGillivray und Seelye, 1950; Rochelmeyer, Stützel und Chen, 1944). An dem Aufbau der Kohlenhydratkomponenten der Soladulcine sind neben D-Galaktose und D-Glukose sowohl D-Xylose als auch L-Rhamnose beteiligt. Das Soladulcin-Gemisch besitzt wie die Leptine (Kuhn und Löw, 1957) eine hohe Larvenwirksamkeit (Buhr, Toball und Schreiber, 1957).

Diese Übersicht zeigt, dass zahlreiche natürliche Inhaltsstoffe der Solanaceen, vor allem verschiedene *Solanum*-Alkaloide, eine Widerstandsfähigkeit gegenüber dem Kartoffelkäfer bedingen können. Bemerkenswert ist allerdings, dass chemisch so verwandte Alkaloide wie Demissin, Tomatin, das *Acaulia*-Tetraosid, die Leptine und die Soladulcine auf der einen Seite und zum Beispiel α-Solanin, α-Chaconin, Solasonin und Solamargin auf der anderen Seite eine so unterschiedliche Wirkung auf den Kartoffelkäfer ausüben.

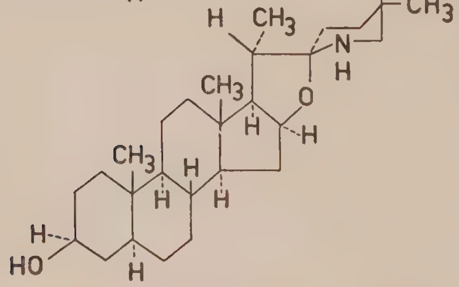
Den oben herausgestellten, chemisch-konstitutiven Merkmalen der wirksamen Alkaloide — Tetrasaccharidkomponente, Xyloseanteil und gesättigtes Aglykon — kann unseres Erachtens jedoch nur eine formelle Bedeutung beigemessen werden, denn die besondere chemische Konstitution der wirksamen Verbindungen steht sicherlich nicht unmittelbar mit der Resistenzausbildung in Verbindung. Wir sind vielmehr der Meinung, daß biophysikalische Vorgänge, wie sie bereits von DETHIER (1956) und anderen Forschern zur Deutung von Geruchs- und Geschmackserscheinungen bei Insekten herangezogen wurden, auch für die spezifische Wirksamkeit der *Solanum*-Alkaloide und somit für die Ausbildung der Kartoffelkäferresistenz verantwortlich zu machen sind.



XVI Solasodin
Δ^5-Solasodenol-(3β)



XVII Soladulcidin
5α-Solasodanal-(3β)



XVIII Tomatidin
5α-Tomatidanol-(3β)

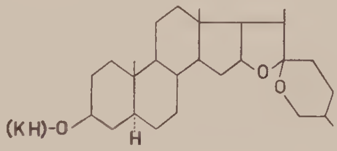
Durch die chemisch-konstitutiven Besonderheiten der wirksamen Alkaloide wird die „Polarität" der Moleküle erhöht, und zwar sowohl durch die Tetrasaccharidkomponente im Vergleich zum Trisaccharid und durch den Xyloseanteil im Vergleich zum Rhamnoseanteil, als auch durch das gesättigte Aglykon im Vergleich zum ungesättigten. Wir verstehen hier unter „Polarität" den intramolekularen

Gegensatz zwischen hydrophilen und hydrophoben Molekülteilen. Je größer dieser Gegensatz ist, desto stärker tritt die Kartoffelkäferwirksamkeit dieser Alkaloidglykoside in Erscheinung.

Diese Überlegungen stehen mit den Ergebnissen der Untersuchung der Oberflächenaktivitäten der Alkaloide im Einklang. Eine erhöhte Oberflächenaktivität macht sich besonders in der vergrößerten Schaumkraft, Benetzungs- und Emulgierfähigkeit der wässrigen Lösungen bemerkbar, und sie ist bei den Tetraosiden aufgrund der erhöhten Polarität ihrer Moleküle größer als bei den larvenunwirksamen Triosiden.

Derartige Oberflächen- bzw. Grenzflächenerscheinungen, die auch für die Höhe der Oberflächen- bzw. Grenzflächenpotentiale von Bedeutung sind (vgl. KIMOTO, 1953 und 1954), könnten darüber hinaus natürlich mannigfache Permeabilitäts- und weitere zellphysiologische Vorgänge beeinflussen. Ob hierdurch nun eine allgemeine Beeinflussung des „chemischen Sinnes" des Insektes möglich ist, und zwar insbesondere in Richtung einer geschmacksbedingten Abschreckung, bedarf einer weiteren experimentellen Nachprüfung.

Bekanntlich sind auch Steroidsaponine (XIX), die mit den *Solanum*-Alkaloidglykosiden chemisch und biogenetisch nahe verwandt sind, oberflächenaktiv. Durchgeführte Testungen bestätigten unsere Vermutung (SCHREIBER, 1954 a), dass auch Saponine — genauso wie zum Beispiel Demissin und Tomatin — eine zum Teil beachtliche futtervergällende Wirkung auf Kartoffelkäferlarven ausüben können (BUHR, TOBALL und SCHREIBER, 1957).



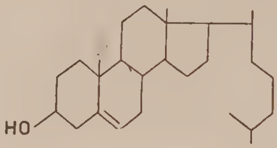
XIX Steroidsaponin

Von verschiedenen Seiten wurde gelegentlich beobachtet, dass bei Kartoffelkäfern, die mit larvenresistentem Pflanzenmaterial, also zum Beispiel mit *demissum*- oder *polyadenium*-Blättern ernährt wurden, Entwicklungs- bzw. Fertilitätsstörungen auftraten. Wir möchten diese Erscheinungen auf eine Beeinflussung des Steroidstoffwechsels der Tiere oder auf eine Beeinträchtigung der Resorption der für das Insekt lebenswichtigen Phytosterine durch die wirksamen Alkaloidtetraoside zurückführen.

Die *Solanum*-Alkaloidglykoside sind — wie die Saponine — hämolytisch aktiv, besitzen also eine Affinität zum Blutcholesterin (FISCHER, 1927; FISCHER und THIELE, 1929; KÖNIG und STAFFE, 1953; VELDSMAN und LOUW 1949; FONTAINE, SCHAFFER, DOUKAS, SCOTT, MA, TURKOT, DEEDS, WILSON und DOOLITTLE, 1955). Darüber hinaus wurde beobachtet (WINDAUS, 1909; SANDER, 1956; SCHULZ und SANDER, 1957), dass diese Alkaloide — und wiederum in besonderem Maße die käferwirksamen — wie zahlreiche Saponine auch in vitro aus alkoholischer Lösung mit Cholesterin schwer lösliche Fällungen geben. Es kommt zur Bildung von Molekülverbindungen (Molverhältnis 1 : 1), die man zum Beispiel als Cholesterin-Tomatid, -Demissid usw. bezeichnen könnte. Bemerkenswert ist, dass nach eigenen Untersuchungen aus schwach saurem Milieu nur die käferwirksamen Tetraoside, nicht aber die Trioside mit Cholesterin eine Fällung geben.

Cholesterin (XX) gehört zur Gruppe der Zoosterine, jedoch werden auch die in Pflanzen vorkommenden Phytosterine, die wie das Cholesterin eine 3β-ständige Hydroxylgruppe besitzen, zum Beispiel die Sitosterine, Stigmasterin usw., durch Saponine und Alkaloidglykoside gefällt.

Aus der Literatur ist bekannt (vgl. SEDEE, 1956), dass Insekten für ihre Entwicklung Steroide benötigen. Während sie im allgemeinen in der Lage sind, neutrale Fette aus andersartigen Nährstoffen zu bilden, kommt ihnen diese Fähigkeit hinsichtlich der Steroide, vor allem der Sterine, nicht oder in nur untergeordnetem Maße zu. Das Insekt ist darauf angewiesen, seinen Steroidbedarf aus der Nahrung zu decken.



XX Cholesterin

Von zahlreichen Arbeitskreisen (BERGMANN und LEVINSON, 1954; BLOCH, LANGDON, CLARK und FRAENKEL, 1956; BRUST und FRAENKEL, 1955; LEVINSON und SILVERMAN, 1954; NOLAND 1954 a und b; SILVERMAN und LEVINSON, 1954) wurden in den letzten Jahren Untersuchungen durchgeführt, um die Beziehungen zwischen der chemischen Konstitution dieser Steroide und ihrer wachstumsregulierenden Fähigkeit bei Insekten zu klären. Diese Arbeiten führten bei einigen Insektengruppen zu dem Ergebnis, dass insbesondere ungesättigte Steroide, zum Beispiel $\triangle^5$-Steroide, einen positiven Wachstumseffekt besitzen. Fehlen diese Steroide, so tritt eine Entwicklungsverzögerung auf. Gesättigte Steroide sind im allgemeinen nicht in der Lage, diese Entwicklungsbeeinträchtigung aufzuheben, im Gegenteil, sie bewirken zusätzlich eine wesentliche Wachstumshemmung. Das Insekt ist vermutlich nicht imstande, gesättigte Steroid-Ringsysteme zu dehydrieren.

Auch die *Solanum*-Steroidalkaloide Demissidin (VI) und Tomatidin (XI) besitzen ein derartiges gesättigtes Ringsystem. Es wäre somit denkbar, dass die ringgesättigten Steroidalkaloidtetraoside vom Typ des Demissin und Tomatin neben der oben erwähnten „Blockierung" der Phytosterine zusätzlich eine hemmende Wirkung auf den Kartoffelkäfer ausüben.

SUMMARY

A summary report is given of naturally occurring substances in plants which confer resistance to the Colorado potato beetle (*Leptinotarsa decemlineata* Say). Alkaloid-glycosides occurring in many *Solanum* and *Lycopersicon* varieties are discussed in some detail, and results are reported of some recent investigations of the alkaloids in *Solanum dulcamara, S. nigrum, S. polyadenium* and *Acaulia.* An attempt is made to explain the mechanism by which these substances affect insects. The harmful effects of alkaloid-tetraosides of *Solanum* on the taste of fodder are attributed to biophysical processes, particularly to surface and interface phenomena. In addition it is assumed that the alkaloids in question induce a blocking of the steroide metabolism and affect the resorption of the phytosterines that are indispensable for the insects, thus explaining frequently observed disturbances in development and fertility.

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DISKUSSION

M. TORKA, J. DE WILDE, H. J. TOXOPEUS, H. J. DEN HERTOG, K. SCHREIBER.

Von WALL und Mitarbeitern[1]) wurden kürzlich aus *Solanum tuberosum* β-Sitosterin und Stigmasterin isoliert, und es ist anzunehmen, daß auch in weiteren knollentragenden *Solanum*-Arten diese Phytosterine bevorzugt vorkommen. Auch β-Sitosterin und Stigmasterin werden — wie Cholesterin — in vitro durch die Alkaloidglykoside Demissin und Tomatin gefällt. Eine „Blockierung" dieser Sterine für den Kartoffelkäfer durch das im Überschuss vorhandene Alkaloid tritt jedoch voraussichtlich erst bei der Zerstörung der pflanzlichen Zelle, zum Beispiel während des Fraßaktes, ein. Die Hauptwirkung der resistenzbedingenden Glykoalkaloide auf das Insekt wird aber in der biophysikalisch zu deutenden Beeinflussung der „Geschmacksvorgänge" zu sehen sein.

Es wird darauf hingewiesen, dass in speziellen Fällen die biologische Wirksamkeit einer Substanz durch Glykosidierung erhöht werden kann. So konnte auch von THORSTEINSON festgestellt werden, dass Sinigrin auf *Plutella maculipennis* stärker wirksam ist als Allylisothiocyanat.

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[1]) SCHWARTZ, J. J. u. M. E. WALL (1955), Steroids and steroidal sapogenins. XXIX. Isolation of the sterols of the white potato. *J. Amer. chem. Soc.* 77 : 5442—5443.

Untersuchungen über die Frage, ob im Verlauf der Vegetationsperiode der Pflanzen neben quantitativen Unterschieden auch qualitative Veränderungen im *Solanum*-Alkaloidgehalt auftreten, wurden bisher kaum durchgeführt. Die gelegentlich gemeinsam mit den Tetraosiden Demissin und Tomatin in geringen Mengen auffindbaren Trioside β-Demissin bzw. β-Tomatin scheinen nicht zu einem bestimmten Vegetationszeitpunkt bevorzugt aufzutreten.

Das Vorkommen resistenzbedingender Tetraoside kann papierchromatographisch leicht erkannt werden. Diese Methode, die unter standardisierten Bedingungen auch eine gewisse quantitative Auswertung erlaubt, kann in der züchterischen Praxis ohne wesentliche Schwierigkeiten auch als Serienanalyse angewendet werden.

Ent. exp. & appl. 1 (1958): 38—40. *North-Holland Publishing Co., Amsterdam*

DIETETIC REQUIREMENTS AND INTERMEDIARY PROTEIN METABOLISM OF AN INSECT *(CALLIPHORA ERYTHROCEPHALA* MEIG.)

BY

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SUMMARY

Calliphora erythrocephala commonly known as the Bluebottle belongs to the Diptera. The larvae of this insect feed on food which is heavily infected with bacteria. Unlike most other terrestrial insects which excrete uric acid, the larvae of *C. erythrocephala* excrete ammonia, the most toxic end product of nitrogen metabolism. In this direct excretion of ammonia the larvae therefore behave like many aquatic animals. Under natural conditions the larvae grow very rapidly. After a growth period of six or seven days they become mature, stop feeding, migrate to a dry place and then pupate.

Although the larvae of *C. erythrocephala* usually live in an environment heavily contaminated with bacteria, it is possible to rear the larvae from the egg under aseptic conditions. When reared on adequate diets the aseptic larvae grow as well as those under natural conditions and metamorphose into normal adult flies.

As in its mode of feeding and living the larvae of the Bluebottle are extreme specialists, it was to be expected that these specialisations may influence its dietetic requirements and intermediary metabolism. In how far these expectations came true was studied in a series of experiments in which the larvae were reared under aseptic conditions so that the intestinal bacteria could not interfere with the results of the feeding experiments and those of the study of the intermediary metabolism.

DIETETIC REQUIREMENTS. After repeated trials it was found that the larvae could be reared from the egg aseptically on a medium consisting of vitamin-free casein, cholesterol, cystine, B-vitamins and water. Larvae reared on this medium pupated and metamorphosed into normal adults. Any addition of carbohydrates or fats to this medium did not improve their growth. By omitting in turn one of the added B-vitamins, it was possible to determine which of these vitamins are necessary for good growth of the larvae. Among the B-vitamins studied the following are required for good growth and development of the larva of *C. erythrocephala*: thiamin, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, folic acid, choline and biotin. On the other hand the significance of inositol or vitamin B_{12} in the nutrition of the larvae could not be demonstrated. Hereupon a series of experiments was carried out to determine the quantitative B-vitamin requirements. It was shown that the larvae require for their optimal growth approximately the following quantities of B-vitamins per gram of medium: 3 μ g. of thiamin, 3 μg. of riboflavin, 35 μg. of nicotinic acid, 4 μg. of pyridoxine, 12 μg. of pantothenic acid, 100 μg. of choline, 0.4 μg. of folic acid and 0.04 μg. of biotin.

With the aid of media containing either an acid casein hydrolysate or a mixture of pure amino acids as the nitrogen source, the qualitative amino acid requirements were studied. The following ten amino acids were found to be essential for growth of the larva of *C. erythrocephala*: arginine, histidine, lysine, phenylalanine, tryptophane, methionine, leucine, isoleucine, valine and threonine. Dispensable amino acids are: alanine, glutamic acid, aspartic acid, proline, glycine, serine, tyrosine, and hydroxyproline. Furthermore it was found that D-serine is extremely toxic to the larvae of *C. erythrocephala.* The natural form of this amino acid did not exert any toxic effect.

In addition to the compounds mentioned above the larva of *C. erythrocephala* requires cholesterol and minerals for its growth; nucleic acid has a growth promoting action.

The results of these feeding experiments show that the nutritional requirements of the larvae of the Bluebottle are not very different from those of other insects. Like other insects the larvae require only the B-vitamins, whereas vitamin A, C, D, and E are of no importance. The amino acid requirements of the larvae have also been found to be much the same as for other insects.

INTERMEDIARY PROTEIN METABOLISM. The intermediary protein metabolism of the larvae was studied with the aid of the stable isotope of nitrogen (N^{15}). The larvae were reared from the egg on food to which a N^{15} labelled compound was added. A medium consisting of casein, cholesterol, cystine, B-vitamins, and water served as food. After the larvae were grown up, they were killed, hydrolysed and a number of their amino acids isolated. The isotope concentrations in these amino acids were analyzed with a mass spectrometer[1]).

Two experiments were carried out. In the first experiment the rôle of glutamic acid in the metabolism of the larvae was studied (N^{15} labelled glutamate was added to the food). The second experiment was carried out to investigate the utilization of dietary ammonia for amino acid synthesis in the larvae. In this experiment the larvae were reared on a medium to which N^{15} labelled ammonia was added as the nitrate.

The results of the experiment in which N^{15} labelled ammonia was fed, offer definite proof that the larvae of *C. erythrocephala* like the rat are able to utilize dietary ammonia for amino acid synthesis.

From the results of the isotope studies it can be concluded that glutamic acid, alanine, and aspartic acid play a central part in the protein metabolism of the larvae. An intensive breakdown and formation of these amino acids takes place during larval life. The breakdown of these amino acids takes place via an intensive deamination. By means of the resulting α-keto acids the larva is able to build up a considerable reserve of fat for use during the pupal stage. Among the isolated essential amino acids (phenylalanine, lysine, histidine, arginine, leucine and valine), only leucine and valine are involved in the reversible intermediary N transfer reactions. This finding shows that the essential nature of these amino acids is due to the fact that this insect is unable to synthesize the carbon chains of these amino acids. Furthermore it was found that the larva of *C. erythrocephala* has more inert amino acids than the rat. In the rat only two amino acids, lysine and threonine, are not involved in the continuous reversible processes of nitrogen shift, but in the larva of *C. erythrocephala* at least five amino acids do not exchange their amino groups with other amino acids. These amino acids are proline, phenylalanine, histidine, arginine and lysine (threonine was not isolated). The finding that proline, which is a non-essential amino acid, is not involved in these processes is especially interesting. In the metabolism of the rat it has been demonstrated that proline, arginine, glutamic acid and ornithine are mutually interconvertible. It is very doubtfull whether these amino acids are also interconvertible in the metabolism of the larvae. However, more experiments will have to be carried out to give a definite answer to this problem. Finally we may conclude from the results of the isotope studies that phenylalanine, known to participate in transamination reactions in mammals, does not participate in these processes in the larva of *C. erythrocephala.*

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[1]) The N^{15} analyses were carried out by Dr. D. H. W. den Boer, at the laboratory of Organic Chemistry, University of Utrecht.

A detailed evaluation of these experiments is given in: PH. D. J. W. SEDEE: Dietetic requirements and intermediary protein metabolism of the larva of *Calliphora erythrocephala. Thesis Univ. Utrecht* 1956. Ed. Van Gorcum Co., Assen.

DISCUSSION

J. H. STEGWEE : Since it was mentioned, that the larvae of *Calliphora* cannot synthesize the carbon-chains of the amino acids valine and leucine, what is then the mechanism by which the rather considerable amount of N^{15} is incorporated ?

ANSWER : The N^{15} found in the isolated leucine and valine has been incorporated by means of transamination processes between the α-keto analogues of the amino acids and glutamic acid or (and) glutamine.

H. J. DE FLUITER : Can we conclude from your experiments as well from the experiments made by HOUSE and FRIEND that all insect larvae need a certain number of the same essential nutrients, but that the amounts of these nutrients acquired are different in the different species ?

ANSWER : Yes, I believe that all insects require for their growth and development B-vitamins (thiamin, riboflavine, nicotinic acid, pantothenic acid, folic acid, biotin, pyridoxine and choline, and the following amino acids: lysine, histidine, arginine, methionine, isoleucine, leucine, valine, threonine, tryptophane and phenylalanine). In some cases it was found that certain insects do not require certain B-vitamins but in those experiments the synthesizing action of the micro-organisms in the gut or food will be responsible for these results.

On the basis of our present knowledge it seems probable that the quantitative requirements for the B-vitamins do not differ much. About the quantitative amino acids requirements in insects not much can be said since only for the honeybee (DE GROOT) this subject has been studied thoroughly.

J. DE WILDE : Did you try to replace cholesterol by other sterols ?

ANSWER : No, only cholesterol was studied. Since HOBSON studied the nutritive value of different kinds of sterols for the sheep blow fly, no further experiments were made in this field.

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DIE ROLLE DER SPEICHELSEKRETE IM WECHSELVERHÄLTNIS ZWISCHEN TIER UND NAHRUNGSPFLANZE BEI HOMOPTEREN UND HETEROPTEREN

VON

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Im Speichel der Rhynchoten gibt es verschiedene Verdauungsenzyme, die eine weitgehende Anpassung hinsichtlich der Art der Nahrung aufweisen und bei der Verdauung wichtig sind. Zugleich gibt es im Rhynchotenspeichel auch Pflanzenwuchs hemmende Stoffe, Auxine und Viren, die alle Krankheitszustände bei den Nahrungspflanzen verursachen welche ihrerseits die Lebensbedingungen der Schädlinge fördern.

Die zu Stechwerkzeugen umgebildeten Mundteile bilden die ausgeprägteste Baueigenschaft, welche die zu den Homopteren und Heteropteren gehörenden Insekten miteinander verbindet. Man kann ohne zu übertreiben sagen, dass auch in der Biologie dieser Tiere der bezeichnendste gemeinsame Zug in der Art und Weise liegt, in welcher der Saugrüssel und die anschliessenden grossen Speicheldrüsen im Dienst der Nahrungsaufnahme gebraucht werden. Diese vollzieht sich bei sämtlichen Homopteren und Heteropteren prinzipiell auf die Weise, dass die Tiere ihr Stechborstenbündel in das Nahrungsobjekt versenken, enzymhaltigen Speichel in dasselbe absondern und dann die dem Speichel beigemengte oder von ihm aufgelöste Nahrung durch die feinen Gänge der Maxillen in den Verdauungskanal aufsaugen. Kennzeichnend ist hierbei der reichliche Aufwand von Speichel, eine Eigenschaft, die sowohl die räuberischen Formen wie auch die Phytophagen auszeichnet. DAY und IRZYKIEVICZ (1953) haben in Australien mit Hilfe von radioaktivem Phosphor festgestellt, dass die Kohlblattlaus, *Brevicoryne brassicae* (L.), bei der Nahrungsaufnahme in die Nährpflanze eine Speichelmenge absondert, die sich auf etwa 1% der aufgenommenen Nahrung beläuft. Diesem so reichlich in das Nahrungsobjekt abgesonderten Speichel kommt eine Schlüsselstellung in den Beziehungen zwischen den Rhynchoten und ihren Nahrungspflanzen zu. Er vermittelt nämlich erstens die Beförderung der aus der Pflanze aufgenommenen Nahrung in den Verdauungskanal. Anderseits wiederum werden die durch die Nahrungsaufnahme dieser Insekten bei den Pflanzen hervorgerufenen Krankheiten hauptsächlich durch die im Speichel enthaltenen phytotoxischen Stoffe oder die mit ihm in die Pflanze geratenden Viren verursacht.

Obwohl diesen Einwirkungen des Speichels der Homopteren und Heteropteren überall in der Welt jährlich recht beträchtliche Mengen von Kulturpflanzen zum Opfer fallen, sind unsere Kenntnisse über dessen Zusammensetzung noch sehr mangelhaft. Am eingehendsten sind in dieser Hinsicht die hydrolytischen Enzyme studiert worden. Von diesen hat man bei den phytophagen Arten Proteasen, Amylasen, Saccharase, Maltase, Pektinase und Lipasen festgestellt (siehe BAPTIST 1942; GOODCHILD 1952; NUORTEVA 1954, 1956 a und b; SAXENA 1955; ADAMS und MCALLAN 1956; u.a.). Diese Enzyme sind jedoch nicht alle in den Speichelsekreten ein und derselben Art enthalten, sondern kommen gewöhnlich nur zu zweit oder dritt vor. Die enzymatische Zusammensetzung des Speichels variiert von Art zu Art; und wenigstens im Auftreten der Proteasen haben sich auch jahreszeitliche oder entwicklungsbedingte Schwankungen ergeben. So wurde gefunden, dass in den Speicheldrüsen der auf Weizen lebenden Wanze *Eurygaster integriceps* Put. in derjenigen Jahreszeit keine Proteasen vorkommen, in der die Tiere ihre Nahrung aus grünen Teilen der Pflanze aufnehmen. Sobald aber die Wanzen zur Nahrungsaufnahme aus dem heranreifenden Korn übergehen, erscheinen in den Speicheldrüsen solche Enzyme (KRETOVICH, BUNDEL und PSHENOVA 1943; KRETOVICH 1944). Bei der Wanze *Lygus rugulipennis* Popp. hinwieder gibt es Proteasen in den Speicheldrüsen der Larven, aber nicht in denen der erwachsenen Tiere (NUORTEVA 1954 b). In diesem Falle dürfte man annehmen können, dass das Vorkommen der Proteasen mit dem grossen Proteinbedarf der heranwachsenden Larve verknüpft ist, dessen Deckung durch ihre Gegenwart erleichtert wird. Bei der Wanze *Miris dolabratus* L. herrscht indessen das entgegengesetzte Verhältnis, indem bei dieser Art die Proteasen den jüngsten, 1.3—2.5 mm langen Larven fehlen und erst auftreten, wenn die Larven eine Länge von 2.6—3.3 mm erreicht haben und allmählich im Begriff sind, sich zu Imagines zu entwickeln. Interessanterweise finden sich jedoch Proteasen nur in den Speicheldrüsen der Weibchen, nicht aber in denen der Männchen (NUORTEVA 1956 c). Letzteres ist vielleicht ein Hinweis darauf, dass die Rolle der Speicheldrüsenproteasen bei *Miris dolabratus* mit der Eiproduktion verknüpft ist. Hinreichend grosse Proteinmengen stellen ja eine notwendige Voraussetzung zur Bildung entwicklungsfähiger Eier in den Ovarien des Weibchens dar. Die in die Nahrungspflanze abgesonderten Speichelproteasen erleichtern dabei zweifelsohne die Gewinnung von Proteinen.

Erwartungsgemäss zeigt die Enzymzusammensetzung des Speichels bei phytophagen Homopteren und Heteropteren eine gewisse Abhängigkeit von der Beschaffenheit der aus der Pflanze aufgenommenen Nahrung. Hinsichtlich der Tätigkeit der hydrolytischen Enzyme sind die ausschlaggebendsten Unterschiede in der Nahrungsbeschaffenheit davon abhängig, ob das Insekt seine Nahrung aus den Siebröhren der Pflanze oder aus dem Mesophyll der Blätter oder des Stengels aufsaugt. Im Mesophyll liegen die Nährstoffe ja meistens in Form von verhältnismässig grossmolekularen Verbindungen vor, während sie in den Siebröhren ziemlich weitgehend aufgespalten sind. Bei manchen mesophyllsaugenden Zikaden sind in den Speicheldrüsen sowohl Proteasen als Amylasen gefunden worden (SAXENA 1954; NUORTEVA 1954 a, 1956 a). Bei solchen Arten dagegen, die ihre Nahrung aus den Siebröhren saugen, fehlen diese Enzyme. Sie sind natürlich auch gar nicht notwendig, da ja die Pflanze durch die Mobilisierung der Nährstoffe vom Standpunkt der Insekten die vorläufige Verdauungsarbeit bereits geleistet hat.

Da nun im Auftreten der Speicheldrüsenenzyme der Rhynchoten eine deutliche Anpassung an die Art der benutzten Nahrung zu bestehen scheint und da

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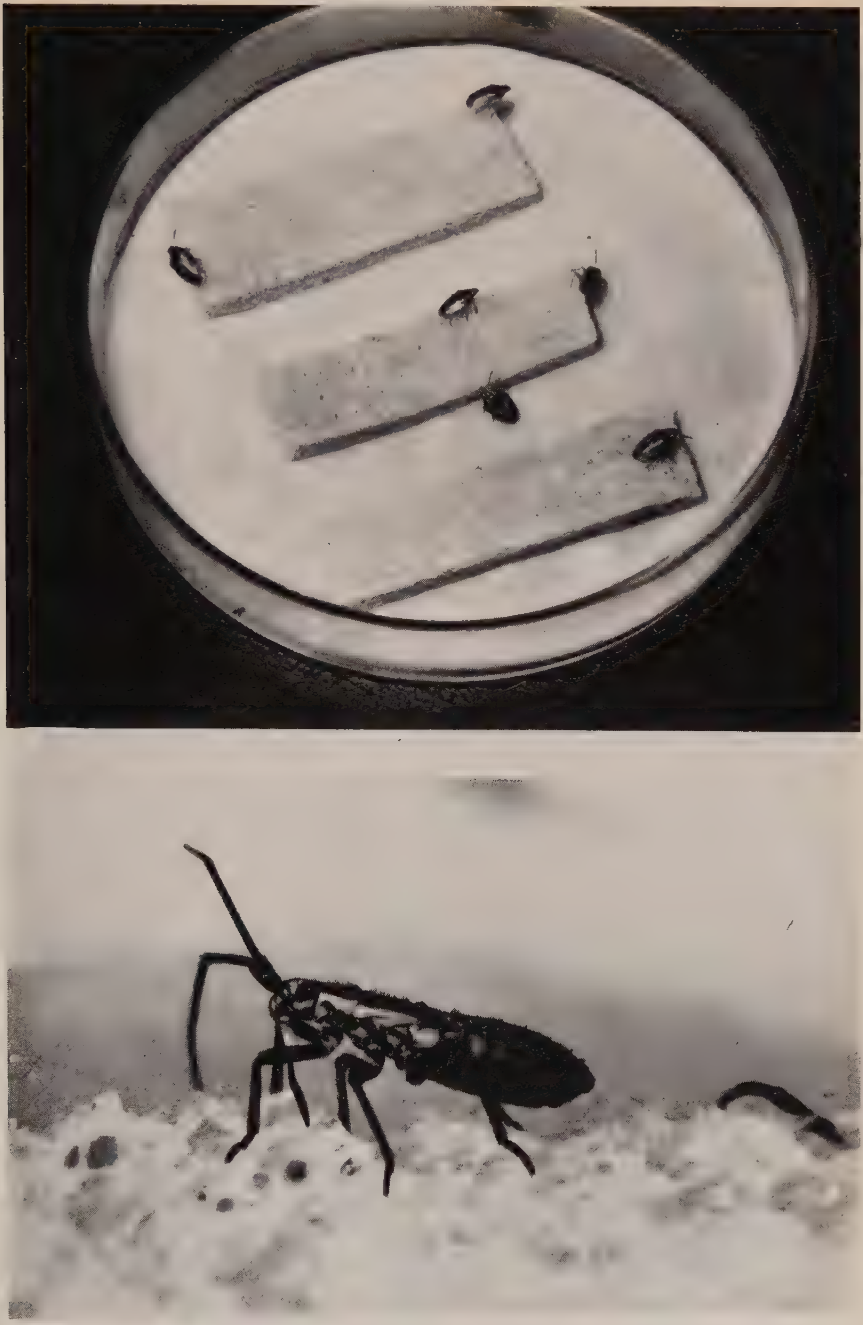

Abb. 1. Die benutzte Methode zur Fütterung von Heteropteren mit synthetischen Nährlösungen. — Man lässt die Nährlösung sich in eine poröse Zellulosemasse aufsaugen und bietet diese dann den Versuchstieren in eine Petri-Schale. — Oben *Aelia acuminata*-Individuen in einer Versuchsschale; unten eine Larve von *Miris dolabratus* beim Saugen an der Zellulosemassa. Aufn.: Teuvo Suominen.

die Enzymsekretion jahreszeitlichen Schwankungen unterworfen ist, war zu vermuten, dass Veränderungen in der Nahrungsbeschaffenheit entsprechende Veränderungen auch in der Absonderung der Enzyme hervorrufen würden. Einige vorläufig unveröffentlichte Ergebnisse meiner Untersuchungen scheinen jedoch zu erweisen, dass das nicht der Fall ist. So konnten bei im Freien gefangenen Wanzen, die allein mit Zuckerlösung gefüttert wurden, keine Veränderungen im Vorkommen der Speicheldrüsenproteasen beobachtet werden. — Diese fanden sich nicht in den Speichelsekreten von Arten, in denen sie von Natur aus fehlen. Andrerseits hörte die Ausscheidung von Proteasen auch bei solchen Arten nicht auf, in deren Speichel sie normalerweise enthalten sind. — Offenbar stellen die deutlich nahrungsbedingte Anpassung der Speichelsekrete und die jahreszeitliche Schwankung ihrer Sekretion phylogenetisch bedingte erbliche Eigenschaften dar, denen die Fähigkeit fehlt, sich bei ausnahmsweise veränderter Nahrung der neuen Lage anzupassen.

Wenn also die Speichelsekrete nicht imstande sind, sich an qualitative Abweichungen in der Nahrungsaufnahme anzupassen, so bedeutet dies jedoch nicht, dass den Bestandteilen der aufgenommenen Nahrung nicht ein Anteil an der Bildung und Beschaffenheit des Speichels zukommen könnte. In manchen Fällen können die Bestandteile der Nahrung als solche in die Speichelsekrete übergehen. So ist bei der Wanze *Plesiocoris rugicollis* Fall. festgestellt worden (NUORTEVA 1955), dass bei Verfütterung mit papain-haltiger Nahrung in den Speicheldrüsen alsbald eine Protease nachgewiesen werden kann, deren pH-Optimum auf der sauren Seite liegt, während bei dieser Art normalerweise nur eine Protease mit leicht alkalischem pH-Optimum zu finden ist. In diesem Falle ist das Papain ganz offenbar ohne Einbusse seiner Wirkung aus der Nahrung in die Speicheldrüsen übergetreten. Es mag manchem sonderbar erscheinen, dass ein Enzymmolekül ungespalten den gesamten Weg aus der Nahrung durch die Darmwandung in die Hämolymphe und dann noch durch die Wand der Speicheldrüse hindurch in den Speichel wandert. Man hat sich jedoch hierbei daran zu erinnern, dass auch so grosse „Proteine", wie das Tabakmosaikvirus, mit seinem Molekulargewicht von 10—20 Millionen, denselben Weg durchläuft. Gleicherweise hat WIGGLESWORTH (1943) gefunden, dass die Hämoglobinmoleküle unversehrt in die Speicheldrüsen einer blutsaugenden Wanzenart gelangen. Weiter verdient erwähnt zu werden, dass eine mit radioaktiver Kohle, Phosphor oder Polonium markierte Nahrung nach Versuchen an Wanzen und Blattläusen aus der Nährpflanze sehr rasch in die Speichelsekrete hinüberwandert (HAMILTON 1935; FLEMION u. Mitarb. 1951, 1952; DAY und IRCZYKIEVICZ 1953; NUORTEVA und REINIUS 1953). — In diesem Zusammenhang hat man auch zu bedenken, dass sich die Insekten von den meisten anderen Tieren und selbst von den Crustaceen dadurch unterscheiden, dass ihnen ein der Leber entsprechendes Organ fehlt, in welchem sich der intermediäre Stoffwechsel abspielen könnte. Angesichts dessen erscheint das Bestreben der Insekten, die aus der Nahrung erhältlichen Wirkstoffe als solche zu verwerten, in gewissem Sinne sogar natürlich.

Die durch den Stich der Rhynchoten bei der Wirtspflanze hervorgerufenen Krankheitssymptome äussern sich meistens in Form von verschiedenen Wachstumsstörungen, wie Gallenbildungen, Verkrümmungen, vorzeitigem Abfallen der Früchte und gesteigerter Wurzelbildung. Diese Symptome erinnern sehr an diejenigen, denen man in Verbindung mit verschiedenen wachstumshormonalen Störungen begegnet; und daraus ist gefolgert worden, dass der Speichel der Rhynchoten auch Auxine enthielte. Man hat zur Stütze dieser Auffassung auf mehrere Tatsachen hinweisen können. So hat der Amerikaner Martin (1930) durch Einspritzung eines aus homogenisierten Zikaden bereiteten Extrakts an den Versuchspflanzen Stengelgallen hervorgerufen. Link u. Mitarb. (1940) wiederum haben beobachtet, dass aus gewissen Blattläusen bereitete Ätherauszüge Haferkoleoptilen zu zusätzlichem Wachstum stimulierten. Dem Franzosen Nysterakis (1946, 1947, 1948 a—c) ist es schliesslich gelungen, aus Blattläusen der Gattungen *Phylloxera, Pemphigus* und *Anuraphis* β-Indolylessigsäure (Heteroauxin) zu isolieren. Ebenso hat er zeigen können, dass die durch diese Blattläuse bei den Pflanzen hervorgerufenen Krankheitssymptome jenen entsprechen, die sich mit Hilfe von Heteroauxin hervorbringen lassen. Besonders interessant ist die Feststellung von Nysterakis vom Jahr 1946, wonach blattlausresistente Weinrebensorten amerikanischen Ursprungs gegen die Wirkung der Auxine nicht so empfindlich sind wie blattlausanfällige europäische Sorten. Nach seiner Auffassung gründet sich die Blattlausresistenz der amerikanischen Weinreben gerade darauf, dass ihre Gewebe auf das Heteroauxin, das ihnen durch die Blattlausstiche zugeführt wird, nicht mit Gallenbildung reagieren.

Alle diese Untersuchungen gründen sich auf Auxinbestimmungen aus homogenisierten Zikaden oder Blattläusen und ergeben darum keinen bindenden Beweis dafür, dass die Auxine auch in den Speicheldrüsen und ihren Sekreten enthalten wären. Im vergangenen Sommer untersuchte ich darum (Nuorteva 1956 b) die Wirkung von Speicheldrüsen-Extrakten von sechs Wanzen-Arten und einer Blattlaus-Art auf das Wachstum von Erbsen. Auxine wurden in keinem einzigen Falle festgestellt. Dies ist natürlich immer noch kein absoluter Beweis für das allgemeine Fehlen von Auxinen in den Speichelsekreten der Rhynchoten, wohl aber zeigt das Ergebnis, dass sie jedenfalls nicht häufig vorkommen. Dagegen stellte es sich bei diesen Untersuchungen heraus, dass der Speichel dreier Arten, nämlich von *Miris dolabratus* L., *Dolycoris baccarum* L. und *Cinara piceae* (Panz.) Auxin-Inhibitoren oder jedenfalls Stoffe enthielt, die das Wachstum der Pflanzen hemmten. Ein Vorhandensein solcher Inhibitoren wurde auch in Amerika von Fischer u. Mitarb. (1946) in der Wanze *Lygus oblineatus* Say entdeckt. Es gelang diesen Forschern auch, den durch die Wanze an der Nährpflanze hervorgerufenen Krankheitszustand durch Hormonbehandlung zu heilen.

Es könnte bisher noch nie bewiesen werden, dass Insekten oder andere Tiere imstande wären, in ihrem Körper Auxine zu synthetisieren. Darum erscheint es ganz natürlich anzunehmen, dass die im Rhynchotenspeichel möglicherweise vorhandenen Auxine aus den Nahrungspflanzen stammen. Bei meinen ersten Versuchen zur Klärung dieser Frage (Nuorteva 1956 c) hat es sich gezeigt, dass bei Fütterung der Wanze *Stenodema calcaratum* Fall. mit heteroauxinhaltiger Zuckerlösung ihr Speichel bei Erbsen gesteigertes Wachstum hervorruft. Bei *Dolycoris baccarum* dagegen ist eine solche Wirkung nicht zu bemerken. Nun gehört aber diese Art zu denjenigen, in deren Speichelsekreten Auxin-Inhibitoren gefunden wurden (siehe oben), und darum ist ein solches Ergebnis bei ihr durchaus verständlich.

Die obige Beobachtung an *Stenodema calcaratum*, dass aus der synthetischen

Nährlösung Heteroauxin in die Speicheldrüsen hinüberwandert, dürfte die Annahme rechtfertigen, dass dies auch in der Natur geschieht, wenn in der Pflanze Auxine in genügender Menge vorhanden sind. Die Tatsache, dass in den Speicheldrüsen der im Freien zur Untersuchung gefangenen Exemplare keine Auxine gefunden wurden, mag als Hinweis darauf gelten, dass es solche Umstände nur selten gibt; — wahrscheinlich wohl nur in einer ganz bestimmten Entwicklungsphase der Pflanzen.

Oben ist in verschiedenen Zusammenhängen festgestellt worden, dass sowohl Papain wie Heteroauxin bei den Rhynchoten unbeeinträchtigt aus der Nahrung in die Speicheldrüsen wandern können. Dies bedeutet, dass wenigstens ein Teil der vom Tier in die Pflanze abgeschiedenen toxischen Substanzen als solche ursprünglich aus der Nahrungspflanze stammt. Die Insekten haben sie wahrscheinlich in ihren Speicheldrüsen nur in eine so hohe Konzentration gebracht, dass sie beim erneuten Eintritt in die Pflanze durch den Stich des Tieres bei dieser einen Krankheitszustand hervorrufen. Zumal was die Auxine betrifft, ist ihre Wirkungsweise bekanntlich völlig von ihrer Konzentration in den pflanzlichen Geweben abhängig. Für die Auffassung, dass die mit dem Speichel in der Pflanze abgeschiedene Toxine als solche von den Nahrungspflanzen herstammten, spricht auch die Feststellung von CARTER (1951) auf Hawaii, dass die Stärke der von der Schildlaus *Pseudococcus brevipes* Ckl. auf der Ananaspflanze hervorgerufenen Krankheitssymptome weitgehend davon abhängig ist, welcher Nährpflanze sich das Tier vor der Übersiedlung auf die Ananas bedient hat.

Die Rolle der in den Speichelsekreten der Rhynchoten enthaltenen phytotoxischen Stoffe und Viren ist als pflanzenpathologischer Faktor in recht vielen Untersuchungen behandelt worden. Weit weniger hingegen hat man sich ihrer Bedeutung vom Standpunkt dieser Insekten selbst zugewendet. Den Speicheldrüsenenzymen kommt unzweifelhaft ein nicht geringer Anteil an den Verdauungsprozessen zu. Dazu sind sie aber, gleichwie die übrigen pathogenen Bestandteile und Mikroorganismen des Speichels indirekt von Bedeutung. Vom Standpunkt der Rhynchoten bedeutet der in der Pflanze entstehende Krankheitszustand nämlich eine Veränderung ihrer Eignung als Nährpflanze. — Man hat nachweisen können, dass die Eignung der Pflanze als Nahrungsquelle für die Homopteren vor allem von der Menge der in den Siebröhren wandernden Nährstoffe — vor allem der stickstoffhaltigen — abhängt (vgl. z.B. LINDEMANN 1948; KENNEDY und BOOTH 1951; NUORTEVA 1952; FENNAH 1953; KENNEDY 1953). Die Nährstoffströmung wiederum ist in Verbindung mit der Mobilisation und Demobilisation der Reservenährstoffe am intensivsten, m.a. W. dann, wenn im physiologischen Zustand der Pflanze Veränderungen vor sich gehen. Dies ist zweifelsohne auch bei Krankheitszuständen der Pflanze der Fall. Dadurch, dass die Insekten durch ihre Speichelsekrete die Pflanze in einem krankhaften Zustand versetzen und so eine lebhaftere Saftströmung in den Siebröhren verursachen, fördern sie zugleich offenbar nicht wenig auch ihre eigenen Möglichkeiten zum Nahrungsbezug. Dies kann nicht ohne Bedeutung sein für das Gedeihen solcher Arten, die sich lange auf ein und demselben Pflanzenindividuum aufhalten oder die auf begrenzter Fläche in sehr dichten Populationen leben. Auch KENNEDY (1951) hat den günstigen Einfluss der Blattgallenbildung sowie der pflanzlichen Virosen auf das Gedeihen der Blattläuse aufgezeigt und ähnliche Beobachtungen sind auch an anderen Rhynchoten gemacht worden.

Auf Grund des oben Ausgeführten findet man, dass die Beziehungen der Rhynchoten zu ihren Nahrungspflanzen auf recht mannigfache Weise durch die Speichelsekrete dieser Tiere vermittelt werden. Erstens ist festzustellen, dass die in den Speichelsekreten enthaltenen hydrolytischen Enzyme bei der Nahrungsaufnahme und Verdauung der Rhynchoten eine wichtige Rolle spielen und eine weitgehende Anpassung hinsichtlich der Art der zu Gebote stehenden Nahrung aufweisen. Anderseits wieder verursachen die Enzyme, Auxine und Viren des Speichels Krankheitszustände der Nahrungspflanzen, welche ihrerseits wieder die Lebensvoraussetzungen der Schädlinge fördern. Weiter scheint es, als ob wenigstens ein Teil der im Rhynchotenspeichel enthaltenen phytotoxischen Substanzen als solche von den Pflanzen herrühre. — Man darf wohl sagen, dass die Zusammensetzung des Speichels bei den Rhynchoten einem Spiegel gleicht, aus dem man die wesentlichen Besonderheiten des nutritiven Verhältnisses der jeweils in Frage stehenden Art zu ihrer Nahrungspflanze ablesen kann. Vom Standpunkt der Erzeugung resistenter Kulturpflanzen hat man hierin einen Hinweis darauf zu erblicken, in welche Richtung die Veredlungsarbeit zu leiten wäre, um die Pflanzen als Nahrungsquelle für den Schädling weniger zusagend zu machen.

Da die mechanische Schädigung der Pflanze durch die Rhynchoten nahezu bedeutungslos ist, lässt sich eine hinreichende Restistenz gegen diese Insekten auch auf die Weise erzielen, dass man der Pflanze eine Widerstandskraft gegen die Toxine des Speichels anzüchtet, ohne dass die Pflanze an sich als Nahrungsobjekt untauglich würde. Diese Möglichkeit, bei Kulturpflanzen eine Toxinresistenz hervorzurufen, besteht namentlich im Hinblick auf die Rhynchoten. Im allgemeinen ergeben sich für die Resistenzzüchtung nur zwei Wege — entweder macht man die Pflanze als Nahrungsobjekt für den Schädling ungeeignet (durch Herabsetzen des Nährwertes, Erhöhen der Giftigkeit oder Herausbildung mechanischer Hindernisse für die Nahrungsaufnahme der Insekten) oder man unterbricht die Kette der Instinkthandlungen, die das Insekt zu seiner Nahrungspflanze hinführen.

SUMMARY

The above article is a short survey of the relation between the salivary secretions of the Hemiptera and their host plants.

In analyses of the composition secretions in the salivary glands of phytophagous Homoptera and Heteroptera, the most extensive work has been done on the digestive enzymes. Proteases, amylases, saccharase, maltase, pectinase and lipase have been detected, but most commonly only 2—3 of these occurs in any one species. An adaptation of the enzyme complement to the diet is evident and in this respect the feeding site used by the insect is especially decisive to the enzyme composition of the saliva. Proteases and amylases occur in the saliva of mesophyll feeders, but are absent in phloem feeders, for which they are useless because, from the insect's point of view, the food is already digested. The adaptation of the salivary enzymes to the nature of the food seems to be largely inherited, for a diet containing nothing but sucrose does not induce adaptive changes in the enzyme content of the salivary glands. By contrast, papain seems to be transferred from a synthetic food to the salivary glands.

The disease symptoms caused in plants by the salivary toxins of Homoptera and Heteroptera have many similarities to those caused by abnormal amounts of growth hormones. Indole-tri-acetic acid has been detected in extracts of crushed aphids and leafhoppers, although no growth stimulating hormones have been detected in salivary glands dissected from

several heteropterous bugs and one aphid. On the contrary, substances inhibiting plant growth exist in the salivary glands of many Heteroptera and in at least one aphid. In some experiments with a heteropterous bug, indole-tri-acetic was observed to have been transferred from a synthetic diet to the salivary glands. It seems possible that auxins, enzymes and other phytotoxic substances occurring in the salivary glands of insects may at last in some cases originate from the host plant and are not produced by the insect.

The salivary enzymes without doubt play an important role in the digestion of the insects secreting them. They are important also in the differentation of Homoptera and Heteroptera to different modes of life. The auxins or auxin inhibitors, as wel as all phytotoxic substances in the salivary glands of Homoptera and Heteroptera are significant for these animals by changing the physiological state of the host plant in such a direction that its suitability as a source of food increases.

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DISKUSSION

W. ROEPKE : Betreffs der im Vortrage zitierten Auffassungen von NYSTERAKIS sei bemerkt, dass gerade bei der amerikanischen Rebe durch den *Phylloxera*-Stich Blattgallen entstehen, während die Wurzeln nicht nennenswert geschädigt werden. Bei der europäischen Rebe entstehen keine Blattgallen, so dass man das Vorhandensein von Abwehrstoffen annehmen muss, die entweder die Läuse am Stechen und Saugen verhindern oder die Gallbildung unterdrücken.

ANTWORT : Leider sind mir die ziemlich komplizierten Verhältnisse zwischen den Phylloxeren und ihren Nährpflanzen nicht genügend bekannt, um hier eine Antwort geben zu können. Darum möchte ich nur auf die Argumente hinweisen, die NYSTERAKIS in seiner Publikation von 1946 gibt. Doch bin ich der Meinung, dass die Auffassung dieses Forschers theoretisch betrachtet sehr interessant ist — selbst wenn sie in diesem Falle unhaltbar sein sollte.

H. J. MÜLLER : Ich möchte in diesem Zusammenhang darauf hinweisen, dass nach BÖRNER auf blattresistenten Europäer-Reben keine Gallen, sondern Nekrosen entstehen die zum Absterben der Reblaus führen.

H. J. DE FLUITER : Der Vortragende hat als Beispiel für ein grosses Proteinmolekül, das in Insekten durch die Leibeshöhle nach den Speicheldrüsen transportiert wird, das Tabakmosaik genannt. Das ist m. E. nicht richtig, weil das Tabaksmosaik ein nonpersistentes Virus ist.

ANTWORT : Die Bemerkung ist ganz richtig. Alle von den Aphiden übertragbaren persistenten Viren wie auch die von Zikaden übertragbaren Viren dürften in dieser Hinsicht ein zutreffenderes Beispiel sein. Ich habe das Tabaksmosaikvirus darum genannt, weil es das grösste pflanzliche Virus ist, das nach unserem Wissen durch die Darmwand in die Hämolymphe eines Insekts wandern kann (siehe M. F. DAY & M. J. BENNETTS : A review of problems of specifity in arthropod vectors of plant and animal viruses. 172 pp., Canberra 1954, p. 14).

J. H. STEGWEE : Hat der Vortragende eine Vorstellung über die Herkunft der Hemmstoffe, die in den Speicheldrüsen gewisser Insekten gefunden wurden ?

ANTWORT : Es wäre verfrüht darüber etwas zu äussern. So viel kann man jedoch sagen, dass es sich nicht um Auxine in übergrossen Konzentrationen handelt.

J. H. STEGWEE : Haben Sie einmal Symptome beobachtet, die auf die Wirkung dieser Hemmstoffe zurückzuführen wären ?

ANTWORT : Bisher habe ich die Hemmstoffe nur bei Untersuchungen im Laboratorium gefunden durch Beobachtungen über ihre Wirkungen auf das Wachstum von abgeschnittenen Stückchen etiolierter Erbsenhypocotyle. Darum ist er verfrüht, etwas darüber zu sagen, durch welche Symptome sich diese noch nicht näher bekannten Stoffe an lebenden Pflanzen äußern.

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PHYSIOLOGICAL CONDITION OF THE HOST-PLANT AND SUSCEPTIBILITY TO APHID ATTACK

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Familiar types of physiological variation in aphid host-plants, such as in the pattern of growth or the water economy, have a great influence upon the growth, development and behaviour of aphids, for reasons that we are beginning to understand. They would merit fuller exploitation for the purposes of aphid control.

The profound effect of physiological as distinct from botanical variation in plants, on their susceptibility to aphids, is exhibited in model form by the distribution of *Cryptomyzus ribis* L. over the surface of one Red Currant leaf (*Ribes rubrum* L.). The aphids do not feed on the general leaf surface but are confined to the inside of the raised 'blister' or 'pocket' galls they produce on these leaves. For a long time it seemed almost superfluous to ask why they should be there, since the galling occurs where they feed. The only other suggestions made, referred to incidental, extrinsic factors such as the shade and shelter provided by the gall cavities. In this case as in many others, intrinsic plant-physiological factors were the last to be considered. But in fact they constitute the main reason for the aphids confining their feeding and reproductive activities to the interior of the galls (Figs. 1 and 2; see also KENNEDY, 1951a, 1951b).

In themselves such correlations between plant-physiological condition and aphid susceptibility do not take us far: the analysis is at too superficical a level. But as a point of departure for the agricultural entomologist, they seem to me to offer certain advantages. They provide a series of pointers for work at all the other levels which must concern him, with many possibilities of cross-checking. First, at the level of insect physiology; secondly, back from that to insect ecology, by reference to the known or discoverable patterns of physiological variation in plants in the field; and thirdly, from that in turn eventually to better integration of aphid control (whatever the means) into the rest of agricultural practice, since the physiological performance of the crop plants is the one thing which all agricultural scientists in common are concerned to understand and control.

HOST-SELECTION THEORY AND ITS PRACTICAL CONSEQUENCES

It must be admitted that such a programme would be dismissed as completely unrealistic in the light of current theories of insect host-preference in general. This subject being "the very heart of agricultural entomology" (LIPKE and FRAENKEL, 1956) and the theme of the present symposium, some reference to general theory is called for. The correlation between aphid susceptibility and such

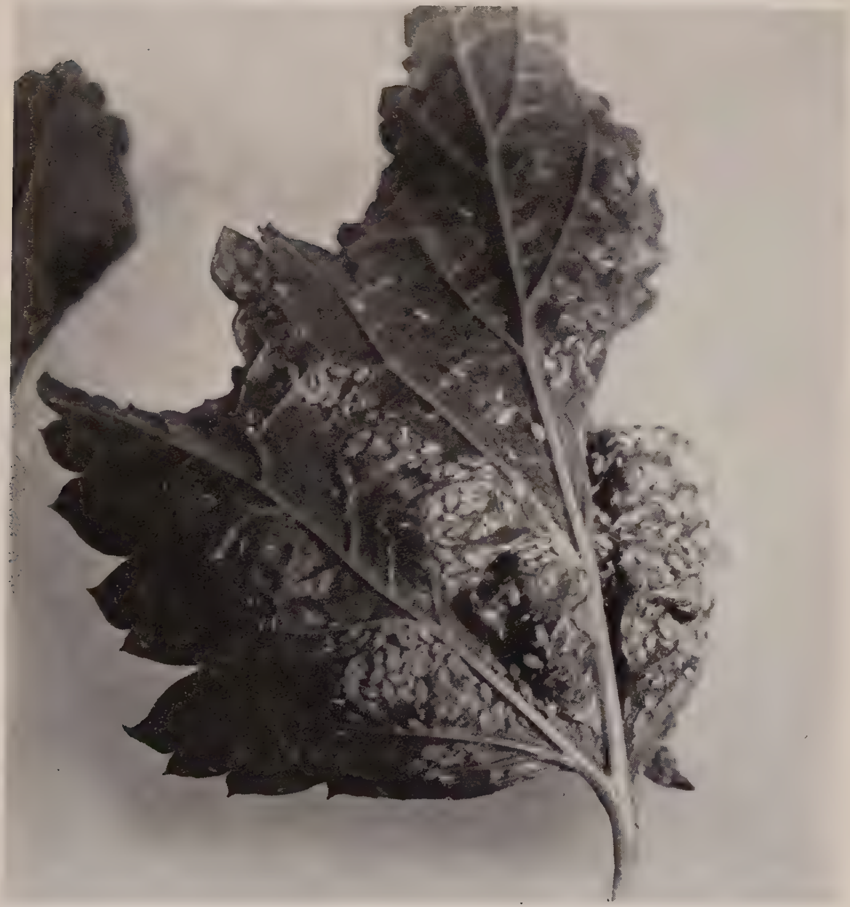

Fig. 1. Distribution of *Cryptomyzus ribis* L. over the abaxial surface of a leaf of *Ribes rubrum* L., with the blister galls everted. See legend to Fig. 2.

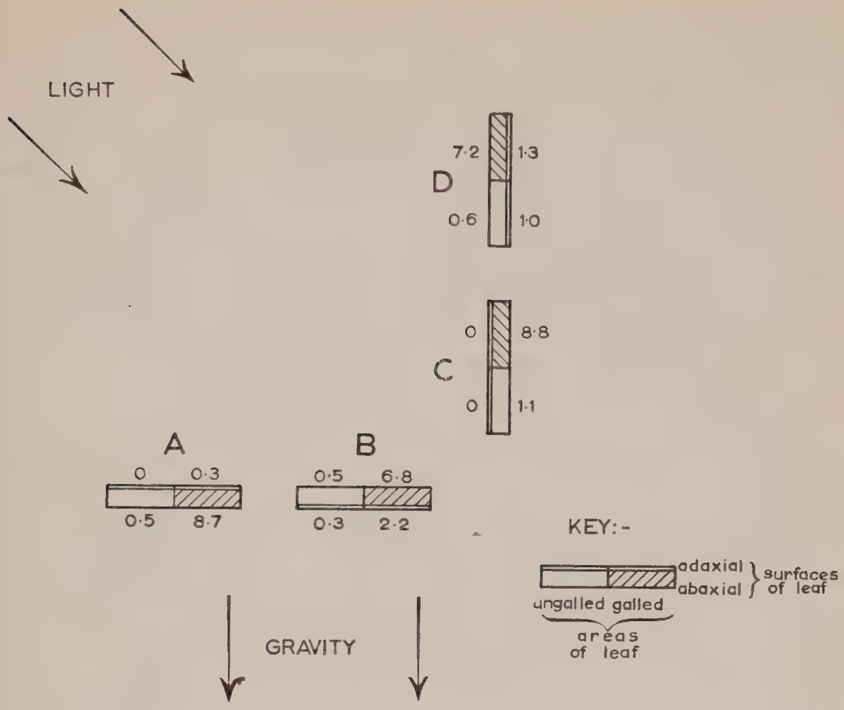

Fig. 2. Feeding preferences shown by *Cryptomyzus ribis* L. between different parts of the leaf surface of *Ribes rubrum* L., after eversion of the blister galls (Fig. 1). Before eversion the aphids were confined to the abaxial, interior surface of the galls, but were removed and released again at random on the leaves and allowed to re-settle before these counts were taken. Based on the distribution of 2079 individuals on 17 leaves with a total area of *c.* 1240 sq. cm. of which the galled regions covered *c.* 376 sq. cm. Eversion of the galls distorted the leaves, so that some parts of each were now abnormally disposed with reference to the light source and gravity (B, C and D), while others parts remained in the normal position (A). All areas of leaf possessing the same combination of intrinsic and extrinsic features were summed, together with the numbers of aphids feeding there, to give the mean densities which are shown in figures against each part of the diagram (aphids per sq. cm). Note the overwhelming preferences for intrinsic leaf features — galled and abaxial surfaces — almost regardless of exposure to light and gravity.

elementary universal physiological processes as leaf growth and senescence, led us some years ago to propose a *dual* discrimination theory of aphid host selection (KENNEDY & BOOTH, 1951; KENNEDY, 1953). This postulated that, in addition to specific stimulatory substances of no nutritive value governing botanical preferences, universal substances such as amino acids which are of fundamental metabolic (nutritional) importance to plants and insects alike, also played a major part in aphid host relations. This of course assumed that the supply of these substances varied greatly according to the physiological activity of the plant. Apart from their "direct" effects on aphid nutrition, we suggested that such common substances also acted as immediate sensory stimuli for feeding and other responses, either in themselves or through token stimuli physiologically associated with them. However, this has been strongly disputed by FRAENKEL (1953a, 1953b; LIPKE & FRAENKEL, 1956) and DETHIER (1947, 1953, 1954), who believe that metabolically unimportant "odd substances" or irregular occurrence among plants, are predominantly responsible for feeding preferences in phytophagous insects: "the curious glycosides, tannins, saponins, alkaloids and essential oils whose occurrence is unexplained by plant physiologists" (LIPKE & FRAENKEL 1956). In support of this, FRAENKEL (1953) collected a large body of evidence from bulk analyses of green leaves, from which he concluded that "sufficient levels of all the major nutrients are present in most, if not all leaves to satisfy phytophagous insects", and doubted whether physiological changes such as leaf ageing altered this situation appreciably. He reminded us that the non-nutrient theory of host selection offers an explanation for the occurrence of all those odd substances in plants, which may have been evolved by the plants specifically as a defence mechanism against insects.

This last is an attractive idea, and one should not overlook other phytophagous organisms here, including eelworms. And as DETHIER (1954) says, "while the recognition of food by nutritionally unimportant token stimuli is certainly well established, the relationship between nutritionally good and sensorially attractive materials remains an open question". But it is plain that DETHIER and FRAENKEL discuss the nutritional-sensory question only because it has been raised; they themselves consider it effectively closed already. The result could be a general disinclination to look for further evidence on the nutritional side: theories are very important at this early stage in the development of the subject.

There are other strictly practical consequences to be faced if these authorities are right that physiological variation in plant constituents (or tokens of them) which are of metabolic importance to both plant and insect, plays little part in determining plant susceptibility to insect attack. This means a definite limitation on our prospects of creating resistant plants. The causes of resistance must then be a highly specialized private problem of entomologists and chemists unaided by the other agricultural sciences. It will remain a special problem for the plant breeder in his turn, always called upon to incorporate a resistant factor which has no known relation to the familiar plant-physiological properties which it is his special skill to preserve and improve. It has been worth while tackling the two very serious potato pests, *Leptinotarsa* and *Heterodera*, in that way, but how many other pests would merit it ? And this kind of resistance based on substances which merely inhibit attack on an otherwise perfectly suitable plant, is just the kind to which the pest itself can be expected most easily to develop "resistance": with the appearance of a new strain which "requires the former repellent (now attractant) stimulus to induce feeding" — exactly as LIPKE and FRAENKEL (1956) suppose host specificity to have evolved in the past.

Fortunately, the nutritional question still remains open. We are not yet obliged to work on the assumption that resistance must be sought almost entirely in odd substances which are a law unto themselves. There is evidence that ordinary physiological differences can mean important differences in susceptibility to insects (PAINTER, 1951, 1958). There is also ample evidence that some nutrient substances stimulate feeding, and THORSTEINSON (1958) has given us fresh instances including glutamate and vitamins. No one would expect all nutrients to stimulate feeding themselves when other nutrient or non-nutrient token stimuli make this unnecessary. Undoubtedly we require more evidence, especially on plant-physiological variation in nutritiousness and its effects on insect behaviour and nutrition. But for aphids, uniform nutritiousness certainly cannot be assumed on the basis of evidence concerning whole leaves. Aphids do not eat leaves; the only nutrients directly available to them are those dissolved in the sap and in transit from one part of the plant to another. This translocation of solutes is neither constant nor haphazard but patterned by the nature and nurture of each plant. To start from correlations between plant physiology and aphid susceptibility, hoping to carry through the programme outlined above, did not therefore seem to us unrealistic. What follows is an attempt to illustrate this approach by reference to three recent and largely unpublished pieces of work done in our laboratory.

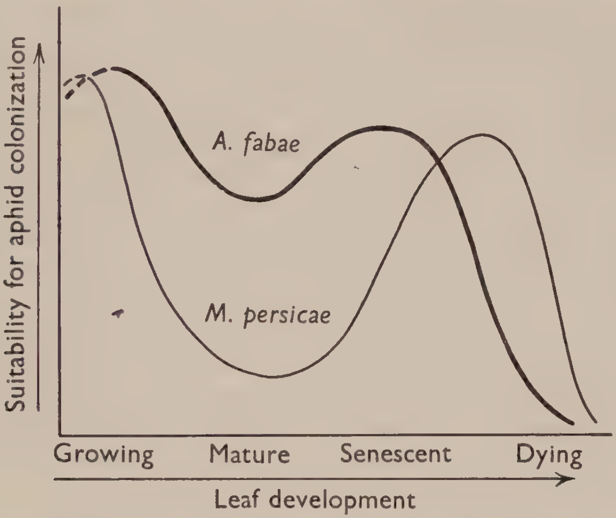

Fig. 3. Schematic representation of the relation between the age of leaves and their susceptibility to colonisation by *Aphis fabae* Scop. and *Myzus persicae* (Sulz.) (from KENNEDY & BOOTH, 1950). In practice the relationship varies greatly, depending among other things upon the rates of leaf growth and senescence, and upon the duration of the intervening mature stage when no growth or senescence is evident in the leaf.

PLANT GROWTH AND SENESCENCE

The starting point here was the observation that growing and senescing leaves of certain plants were more susceptible to *Myzus persicae* (Sulzer) and *Aphis fabae* Scopoli than mature green leaves of the same plants (Fig. 3). Three measures of "susceptibility" were used in the earlier work: simple counts of free infestations, cage measurements of feeding preferences and cage measurements of birth rates. Our hypothesis to account for the leaf-age effects was that the aphids' food (phloem sieve-tube sap) would be especially rich in soluble organic nitrogen compounds of high nutritive value — amino acids and amides — at or near places where growth and hence protein synthesis was going on in the plant, and again where senescence with protein breakdown was going on; but poor in these compounds where neither growth nor senescence was in progress, as in mature green leaves. LINDEMANN (1948) had put forward the same hypothesis in connection with her work on leaf-feeding aphids. We still have no direct evidence that the postulated differences of phloem sap composition exist between leaves (and other organs) at different developmental stages, on the same plant at the same time. And we recognize that part of this hypothesis is at variance with the usual picture of translocation in the plant occurring down a concentration gradient of organic nitrogen and other solutes, from their source in mature green photosynthetic leaves or in storage tissues, to growing organs functioning as "sinks". But that particular plant-physiological question can be left aside for the time being, since MITTLER (1953, 1954, 1957, and in press), following LINDEMANN (1948), has provided some direct quantitative evidence on the main insect-physiological question before us, by comparing plants at different stages of development rather than individual organs.

He used a different aphid, *Tuberolachnus salignus* (Gmelin), which feeds in the phloem sieve tubes in the woody stems of the willow, *Salix* spp. Samples of the aphids' actual food were obtained by collecting the sap exuding from their stylet stumps left embedded in the stem after the rest of the aphid had been cut away (KENNEDY & MITTLER, 1953). This sap was analysed qualitatively and quantitatively, and so was the honey-dew being excreted by intact aphids on the same stems at the same time. The composition of the two fluids was found to vary together. On one stem at one time, the total sugar content of the honey-dew was not appreciably lower than that of the sap, suggesting that little of the sugar was being assimilated (although some of it was chemically altered). But the nitrogen concentration was always lower in the honey-dew than in the sap. This difference, when measured and multiplied by the total volume of sap taken in by aphids feeding and growing on the same stem for a given time, approximated closely to the measured gain of nitrogen in the bodies of the aphids during that time in several detailed experiments. Thus the sap was apparently the only source of nitrogen available to the aphids (cf. TOTH, 1952).

TABLE I

Effect of growth in the host-plant on the feeding activity, growth and development of Tuberolachnus salignus (Gmelin) on stems of Salix for 7 days from birth (from MITTLER, *1954).*

	State of foliage	
	actively growing	mature
Total honey-dew output per nymph, cu.mm.	110	140
Total nitrogen assimilated per nymph, µg.	105	6
Instar reached	III—IV	III
Percentage of alate forms	24	67

Moreover, the nitrogen content of the sap in the stem varied greatly through the cycle of growth, maturity and senescence of the foliage on a tree, as would be expected from the work of plant physiologists (e.g. ZIEGLER, 1956; TAMMES, 1958). And the amount of nitrogen assimilated by aphids feeding on the stems varied correspondingly. For example, Table I summarizes the results of one experiment using two potted willow plants, one in active growth and the other having completed its growth and not yet begun to senesce. New-born aphids were left to feed on the stem of each plant for a week. Ninhydrin tests showed that the concentration of amino-nitrogen was many times greater in sap exuding from cut stylets in the tree with growing leaves than in sap from the tree with mature leaves. Correspondingly, the gain of total nitrogen (also fresh weight and dry weight) by the nymphs during the week was about 17 times greater on the tree with the growing leaves. The output of honey-dew was recorded continuously during the week as a measure of the relative intake of sap. It proved to be consistently rather higher on the mature plant with little nitrogen in the sap. In this case therefore one can say with more than usual confidence that the faster growth of the aphids on the growing plant was due, not to more feeding there, but to better nutrition in the strict sense of the term.

From a number of such experiments, comparing the same plant at different times as well as different plants at the same time, it was possible to build up this general picture. The nitrogen content of the sap was very high as soon as the buds began to swell and the aphids began to feed on the stems. Before that when the buds were still dormant the aphids probed the stems often but would not settle down to feed, so no sap or honey-dew could be obtained for analysis. As shoot growth proceeded and slowed the sap nitrogen content fell; when leaf senescence set in it rose again. The growth rates of aphids feeding on the stems began high, then fell and then rose again in parallel.

So much for nutrition; what evidence was there of sensory stimuli associated with the differences of nutritiousness ? MITTLER was most concerned with nutrition but he did observe both excretory and morphogenetic responses, presumably organized by the central nervous system upon receipt of some sensory stimuli. Thus in the first experiment mentioned above (Table I), the following additional differences were observed on the two plants. Associated with the faster feeding as indicated by the greater daily volume of honey-dew excreted where the sap was poor, the act of excretion was executed more frequently: smaller droplets being expelled, but 2-5 times more often. The aphids also developed more slowly on the poor sap, being about half an instar behind by the end of the week; and more of them turned out to be alates (Table I). LINDEMANN (1948) had earlier shown a correlation between sap nitrogen content and the parturition and wing-development responses, as well as the growth, of certain leaf-feeding aphids : *Cryptomyzus ribis* L., *Neomyzus circumflexus* (= *Aulacorthum* (*Neomyzus*) *circumflexum* (Buckt.)) and *Macrosiphon koehleri* (= *Macrosiphum euphorbiae* Thomas)). Although the sap analysed in this work was not obtained through aphid stylets but from cut stems or as contaminated exudate from petioles, etc., its nitrogen content, the nitrogen content of the aphids' honeydew, the aphids' growth, and aphids' responses, all showed the same relation as MITTLER found to the growth, maturation and senescence of the leaves. These workers' results do therefore appear to support the hypothesis that the host responses of aphids are governed partly by stimuli associated with sap rich in organic nitrogen and possibly even given by that sap itself. It would be surprising if the feeding and parturition preferences observed in *Aphis fabae* between young, mature and senescent leaves were not also governed by such "nutrient stimuli".

At the same time MITTLER's demonstration of 'true' nutritional differences associated with developmental changes in the plant overcomes what was certainly a serious weakness (DETHIER, 1954; LIPKE & FRAENKEL, 1956) in the original case for the dual discrimination theory. We were quite unjustified in using the parturition rate of young adults over a few days as a measure of fecundity and hence of nutrition[1]). Parturition is itself a nervous response to external and internal stimuli no less than is feeding, as subsequent work has shown (JOHNSON, 1953, 1954; MITTLER, 1954; CARTIER & PAINTER, 1956; KENNEDY, 1958). The first offspring produced by an aphid (as by many insects) are not the product of its feeding as an adult but of the food taken in during its immature stages. Indeed, as UICHANCO (1924) first showed, and LAMB (1955) recently confirmed in *Aphis fabae*, embryonic development begins within an aphid before its birth, in the grandmother's body. Hence when we speak of an adult aphid responding to stimuli betokening nutritiousness, it is not only her own nutrition that is meant, but even more the nutrition of the young she deposits in response to those stimuli. The main significance of the host-selecting behaviour of mother aphids is for the next generation, as in other insects.

DETHIER (1954) brings forward this well-known fact that the female often "selects a plant or part thereof which is to feed the larvae and is not at all the proper food for herself", as an argument for the predominantly odd-substance theory of insect host-selection and against the idea that nutrients or tokens of them are important co-stimuli. But nutritional effects always come after the behavioural response, whether that be oviposition or feeding. In this context it is immaterial whether the nutritional effect is on the individual that makes the behavioural response, or on its progeny. Our concern is with the stimuli for the selective response: are some of them linked with nutritiousness or not ?

To take an extreme case, consider the selection of oviposition site by the sexual female of an heteroecious aphid such as *Aphis fabae* Scop. in the autumn. This certainly has nothing to do with food for the ovipositing female herself. Yet it appears to be a "nutritional" selection on her part (although the stimuli are most unlikely to be actual nutrients), for it is related to a change that occurs in the location of food supplies on the plant between her generation and the next. It is not primarily a "botanical" selection. These females do not have to find the right kind of plant, for they are born on it, apterous. They may well have "botanical" preferences (this has not been tested), but the major act of botanical selection is carried out by their mothers, the winged parthenogenetic viviparous *gynoparae* and by the winged males. The special act of selection carried out by the ovipositing sexual female is between parts of one plant, and is fitted to the developmental physiology of the plant. It consists of (i) ceasing to feed on and walking off the leaves which were senescing and therefore suitable for feeding while she was immature, but which will soon become unsuitably old and then fall from the plant, and (ii) moving on to the bark of the woody stems where no suitable feeding sites are now present, and depositing the eggs in crevices especially around the dormant buds which will be growing and suitable for feeding by the time the eggs hatch in the spring.

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[1]). The distinction made by DETHIER (1953, 1954) between active and passive host selection, and the similar one made by PAINTER (1951, 1953, 1958) between preference and antibiosis, presented not as loosely descriptive but as analytical and quasi-physiological, are also rather misleading. For these distinctions imply that while host finding and feeding are active responses to the host, the subsequent effects of the host plant — on parturition or oviposition, on growth, morphogenesis and survival — are not. The working notion of a 'truly' or 'strictly' nutritional effect is also one that begs some fundamental physiological questions. Insects can only grow or ripen their eggs after ingesting certain substances in sufficient amount; but it is to say the least premature to assume that this effect is a passive one, different in kind from other effects of the external and internal environment which are known to be organized responses.

The larger phenomenon of seasonal host alternation (heteroecy) itself seems also to be at bottom a matter of seasonal selection by "nutrient" stimuli in this sense, even though the selections made are between different kinds of plant (LINDEMANN, 1948 ; KENNEDY, 1953). It is the concomitant selection of a certain number of winter and summer hosts as a set, in preference to all the other kinds of plant not colonized at any season by the given species of aphid, that constitutes the true "botanical" selection which we may reasonably ascribe to odd substances. The different forms of one aphid species differ in the relative strength of their 'botanical and 'nutrient' preferences, in accordance with their differing ecological roles (KENNEDY & BOOTH, 1954). The primary (winter) host is preferred to other plants even by the summer forms of the aphid which rarely colonize it in nature; it is their "nutrient" preference which takes them elsewhere.

PLANT WATER RELATIONS

The study of another kind of plant-physiological "nutrient" factor for aphids, physical rather than chemical, started from the correlations observed between aphid behaviour and plant water status. A good deal of seemingly contradictory evidence has been published on the effects of plant water shortage on aphids: unfavourable effects such as the aphids becoming restless or developing wings (e.g. RIVNAY, 1937), and the reverse — mass multiplication in droughts (e.g. MARKKULA, 1953). In neither case has the mechanism of the effect on the aphids been clear. The effects on the plant are known to be complex: loss of turgor, arrest of growth, and hydrolysis in the leaves resulting in premature senescence. During the original experiments on leaf ageing as a factor in the host alternation of *Aphis fabae*, the outdoor results were much less consistent than the greenhouse results using well-watered plants (KENNEDY & BOOTH, 1951). In warm dry weather when the senescent leaves of the outdoor Sugar Beet plants wilted most, they ceased to be preferred by the aphids to the mature leaves. The more rigid leaves of *Euonymus europaeus* L. never wilted visibly outdoors. But discs punched out of them showed that they also lost water and they also became then less acceptable to the aphids. Indeed they became relatively more unacceptable than the beet leaves under similar conditions.

Experimental comparisons were therefore made of leaves on watered and unwatered plants under otherwise identical conditions both outdoors and in the greenhouse using *Euonymus* and *Vicia faba* L. (LAMB, 1955; KENNEDY & BOOTH, unpublished). When the plants were not watered, consistently negative effects were recorded on feeding activity, parturition rate, survival and form (production of alates). Tables II and III exemplify the results obtained. The question then was, what made the water-deficient leaves unfavourable ? Several experiments showed that the effects were readily reversible in the same leaves by restoring the water

TABLE II

Effect of water supply to the host-plant, Euonymus europaeus L., on colonisation by Aphis fabae Scop. in the field. Two similar lots of four potted plants were exposed for 10 days during which lot A was kept watered and lot B left unwatered. After counting and removal of the aphids, the same plants were exposed for a second period of 21 days with the watering treatment reversed, and fresh counts then made. Figures refer to mature leaves only, present through both periods (KENNEDY & BOOTH, *unpublished*).

		Alate mothers per leaf	Progeny per leaf
First period	Lot A watered	1.7	18.5
	Lot B unwatered	0.5	8.0
Second period	Lot A unwatered	0.2	0.7
	Lot B watered	1.9	5.5

TABLE III

Effect of wilting of the host-plant, Vicia faba L., on parturition by apterous Aphis fabae Scop. caged singly on the leaves of potted plants. Mean number of young produced per adult by three adults in three days (from LAMB, *1955).*

	Age of leaf		
State of plant	young	mature	old mature
Turgid	14.7	5.0	2.3
Wilted	5.7	2.3	1.0

supply to the roots. Moreover, aphids caged on all ages of leaf, young, mature and senescent, were affected in the same reversible way at the same time. Thus the negative effects of water shortage cut right across the effects of leaf age, and they could not be related to chemical changes in the leaves (LAMB, 1955). It appeared that the immediate physical effect of loss of turgor might be responsible. In other words the aphids might depend upon the sap pressure to maintain their sap intake, and, in addition, respond actively to changes in it.

It was originally to test this hypothesis that the stylets of feeding aphids were cut, to see if sap would continue to come up without any assistance from the aphid. It did so in a few cases when scissors were used on *A. fabae* on beans, but the stylets here were easily dislodged. MITTLER found that much more repeatable results were obtained when the stylets could be cut guillotine-fashion against a rigid woody stem, and developed this as a routine technique for the work already mentioned. He measured the rate of sap exudation from *Tuberolachnus* stylet stumps in a willow stem, and compared it with the rate of honey-dew excretion by intact specimens feeding on the same stem at the same time, on a number of occasions. The two rates were not significantly different, which confirmed the dependence of the aphids on the sap pressure within the plant, and showed how mistaken we had been in thinking of aphids as "sucking" insects. Furthermore, interrupting the water supply to the stem, or to a small area on it by local incisions down to the wood, caused the exudation from cut stylets to slow down and stop while at the same time intact feeding aphids withdrew their stylets and wandered away — like *A. fabae* from wilting leaves.

Turning from this back to ecology again, it was inferred that the invisible loss of turgor by *Euonymus* leaves in fine summer weather was an additional factor preventing *A. fabae* from colonising them then, reinforcing the effect of the maturity of all the leaves. We obtained some indications that the mature leaves of *Euonymus* developed a day-time water deficit earlier in the season than sugar beet leaves of the same age; and later on in the season when the beet leaves wilted daily but recovered their turgor and water content at night, the *Euonymus* leaves did not regain water at night, being apparently in a chronic state of water deficiency (Cp. WEATHERLEY, 1951, on cotton plants). Secondly, there are indications that the growth and also the senescence of *Euonymus* may be restricted by water shortage in the summer even in our supposedly wet climate. The general absence of growing and senescing leaves on many shrubs and trees in the summer, which we have suggested lies at the root of the phenomenon of host alternation, may not be so fixed a property of such plants as we thought. It may be imposed upon them to some extent by water shortage.

The herbaceous secondary hosts of *A. fabae* on the other hand, bean and sugar beet, manage to continue growing to some extent when the drought is not too severe, apparently at the expense of the old leaves. The leaves start senescing as soon as they cease growing or even before, so that the middle-aged leaves become acceptable to the aphids instead of being truly mature and unacceptable (KENNEDY, IBBOTSON & BOOTH, 1950). The growing leaves are the last to lose water and droop in a drought, while the older leaves which wilt more readily also recover readily, as at night. Continuous wilting of course quickly kills the leaves; but intermittent wilting favours the progressive type of senescence which in turn favours aphids, perhaps because of an increase in translocatory amino-nitrogen (Cp. GATES, 1957, on tomatoes). Leaf senescence brought on in this way may well be involved in the reported cases of drought favouring aphid multiplication. Usually too many other factors are varying at the same time to assess the part played by the condition of the plants. But in a randomized plot experiment with potatoes planted at a succession of dates, TAYLOR (1955) observed that when premature senescence was induced in one of the plantings by a temporary drought, there was a rapid multiplication of *Myzus persicae* on these particular plants only, so that in this case extrinsic factors were probably not responsible.

It is therefore not very surprising that the literature on this subject appears self-contradictory. The plant-physiological consequences of water shortage may, in fact, have contradictory effects on aphids, reducing the quantity of sap they can get but at the same time improving its quality. Clearly we shall not be able to predict or control which way the balance of effect will go in any given case in the field until much more quantitative work has been done. But we should eventually be able to obtain aphid resistance through altering the water economy of the plants, by cultural or plant breeding methods.

VARIETAL RESISTANCE

It is patently absurd to think of trying to create resistance in field crops such as beans by altering their growth pattern to resemble that of *Euonymus*, with no growth or senescence going on in the summer. The present physiological properties of crop plants are, with present techniques, what we require of them as crop plants. This truism might suggest that physiological differences as of growth pattern play little or no part in the observed varietal differences of resistance to aphids. That is often assumed and it fits the odd-substance theory of insect host-relations. But there is some evidence that it is not always true. Thus BALD, NORRIS and HELSON (1946) in Australia and then TAYLOR (1955) in England have compared a number of potato varieties for aphid susceptibility. Both came to the conclusion that the main reason for the observed differences was the different growth patterns of the varieties. For example TAYLOR found that *Myzus persicae* with its marked preference for senescent potato leaves, attained the biggest populations on the variety which developed such leaves first, that is, the earliest variety (Table IV). He found the same correlation between the incidence of senescent leaves and of *persicae* populations, whether he was comparing the same variety planted at different dates, or at the same date in different years, or different varieties planted together.

TABLE IV

Comparative susceptibility of potato varieties to infestation by Myzus persicae (Sulz.) and Aphis nasturtii (Kltb.). Maximum populations per plant in a trial by TAYLOR *(1955). M. persicae shows a marked preference for senescing leaves on potatoes, and in this trial the early-maturing variety Arran Pilot bore a much higher proportion of such leaves than the other varieties, when the aphids arrived. A. nasturtii apterae colonize leaves of all ages, so that the number of leaves available for them is greatest on late-maturing varieties such as King Edward and Majestic.*

Maturation class of varieties:	'First early'	'Second early'	'Maincrop'	
Variety:	Arran Pilot	Ulster Chieftain	King Edward	Majestic
M. persicae	1410	340	920	490
A. nasturtii	270	490	1310	1410

Such information suggests there may be possibilities of increasing aphid resistance physiologically, at any rate by cultural methods (e.g. water control). But since early and late varieties of potatoes are both needed in practice, neither can be eliminated merely to avoid aphid attack; and there might appear to be no prospect of breeding for resistance along these lines. Perhaps that is what BALD *et al.* (1946) meant when they drew the strange conclusion that there was no evidence of aphids preferring any of the varieties they tested. Nevertheless, where aphids seriously limit the growing of a crop, genetical differences in physiological characters have been used for aphid resistance, or so it is believed. It is a general if unproved assumption that Winter (autumn-sown) varieties of Field bean which are less heavily infested and less damaged by *A. fabae* than Spring varieties, derive their resistance from the simple fact that they have out-grown the most susceptible juvenile stage before the aphids arrive (e.g. RADEMACHER, 1939). In this case, it is believed that the grower is employing a genetical difference in one kind of physiological property, frost resistance, to permit earlier planting to obtain another kind of physiological difference, in age, which in turn confers aphid resistance.

But undoubtedly the most interesting problem is presented by varieties which are more alike in their agricultural performance, such as varieties within the general class of Spring beans. Here, if anywhere, one might expect that differences quite unconnected with growth patterns and aphid nutrition would be entirely responsible for any differences of aphid susceptibility that exist. H. TAMBS-LYCHE (unpublished) studied such a case in a small mixed planting of two varieties of Field (Spring) beans: *Rastatt* which is relatively resistant and *Schlanstedt* which is very susceptible to *Aphis fabae* (MÜLLER, 1951, 1953, 1956, 1957). As expected from MÜLLER's extensive studies, the natural incidence of *A. fabae* alatae was consistently lower on Rastatt than on Schlanstedt in repeated counts made throughout the season. This original difference was reflected in the relative size of the colonies which built up on the plants in the intervals between sprayings to kill off all the aphids.

As usual on beans, the majority of feeding alatae were counted in the young furled leaves at the shoot tips and in the crevices between the developing flower buds near the tip. The colonies started by the alatae were still more concentrated into these flower bud crevices. The records kept of the growth, flowering and fruiting of the plants showed that this preferred site among the loosening flower buds became available for colonisation *later* on Rastatt than on Schlanstedt (Table V). For several weeks in the early and most critical part of the season, Rastatt continued to offer fewer such sites for colonisation, because the first flower buds developed on Rastatt about two nodes higher than on Schlanstedt. In this and other respects Rastatt behaved in this trial as a later variety than Schlanstedt, differing from it in the same way as Field beans as a class differ from Broad or Garden beans (CHAKRAVARTY, DRAYNER and FYFE, 1956). Here then was a difference in developmental physiology which did appear to be helping in some measure to make Rastatt more resistant, if only in a rather mechanical fashion as far as the eye could judge.

TABLE V

Comparative growth and development of two varieties of Field bean (Vicia faba L.): Schlanstedt which is very susceptible to Aphis fabae and Rastatt which is relatively resistant. Mean number of organs developed, and final weight yield of beans (seeds), per plant (TAMBS-LYCHE & KENNEDY, *unpublished)*.

		Schlanstedt	Rastatt
May	Leaf-bearing nodes	7.1	7.2
	Inflorescence-bearing nodes	3.5	1.6
July	Leaf-bearing nodes	75	99
	Pod-bearing nodes	33	43
August, when harvested	Filled pods	32	47
	Beans	94	130
	Weight of beans, g.	66	61

However, this obvious difference did not last beyond the first weeks. The flower buds ceased to be preferred as they opened in June, and Rastatt soon bore as many unopened ones as Schlanstedt. By July Rastatt offered more favoured sites than Schlanstedt. Yet the aphids continued to prefer Schlanstedt; and we confirmed MÜLLER's (1953) finding that the individual organs of Schlanstedt were preferred, even the expanded leaves, and not just the plants as a whole. From this one might be tempted to conclude that the sole cause of the preference was some subtle difference between all parts of the plants which was quite unconnected with growth pattern and nutrient supply. Such a conclusion, however, still seems premature when the other aspects of the lateness of Rastatt are considered.

During the period of flowering and fruiting in June and July, Rastatt was engaged in the production of more shoots, more leaves, more flowers (of which many fell without opening) and more pods, per plant, than Schlanstedt (Table 5). The final result at harvesting was more numerous but smaller beans from Rastatt than from Schlanstedt, with about the same total weight yield of beans per plant. There is no reason to suppose that the total assimilatory product of Rastatt was any greater than Schlanstedt's. But it does appear to have been distributed differently. On Rastatt, growth was going on at more points at the same time; hence less at each. It would seem to follow that an aphid feeding in the phloem sieve tubes supplying any one growing organ of Rastatt, say a flower bud, would be getting sap less rich in nutrients than it could get on a similar organ of Schlanstedt.

This is still only a suggestion; but it invites further work in several directions. AUCLAIR and MALTAIS (1950) and MALTAIS (1951) found a lower amino-nitrogen content in pea varieties resistant to the pea aphid, than in susceptible varieties. Since it was not the whole plants but only the tops of growing shoots that they assayed for amino-nitrogen, the question arises whether this resistance, too, may have been due to a small difference of growth pattern. The postulated difference in sieve-tube sap composition between varieties with slightly different growth patterns (Cp. the difference between vegetative and flowering shoots of alfalfa: DAHMS and PAINTER, 1940) could be checked directly, by comparing the composition of sap exuding from cut aphid stylets on the two plants. Again, it would be interesting to see how far Rastatt might lose its resistance if earlier flowering were induced by vernalising the seed (CHAKRAVARITY et al., 1956), and how far Schlanstedt might become more resistant if more "bushing" were induced by destroying the first shoot. The results might be negative and so fail to give the hoped-for hints to the plant breeder. But it was encouraging to find anything worth further investigation from the plant-physiological point of view in this case. The physiological difference between Rastatt and Schlanstedt is not apparently of any other agricultural significance. If it is responsible for the difference in aphid resistance, this is the kind of physiological, nutritional resistance, which could be selected or bred for without losing any other desirable character in the plants.

ZUSAMMENFASSUNG

1. Es ist bekannt, daß unregelmäßig auftretende Sekundärstoffe unbekannter Funktion in den Pflanzen oft die Reize darstellen, mit deren Hilfe die Insekten ihre Wirtspflanzen unterscheiden. Es wird behauptet: I. daß vorwiegend solche Reize für die Wirtswahl verantwortlich seien, II. daß alle für die Insekten notwendigen Grundnährstoffe von fast allen Blättern in ausreichendem Maße geliefert würden und daß deshalb III. die für

den Stoffwechsel der Pflanzen wie der Insekten als wichtig bekannten häufigen Verbindungen eine geringe Rolle als Ursache der Präferenzerscheinungen spielten (sowohl als unmittelbare Reize wie als physiologisch mit ihnen verbundene Signalfaktoren). Die Theorie der „doppelten Unterscheidung" bei der Wirtswahl der Blattläuse, andererseits, fordert, daß solche „Ernährungs"reize eine bedeutende Rolle spielten, zusammen mit spezifisch pflanzlichen Reizen; und daß der Nährstoffgehalt der Pflanzensäfte, von dem sich die Aphiden ernähren, dem physiologischen Zustand der Pflanzen entsprechend, in weiten Grenzen schwankt.

2. Es wird hier darauf hingewiesen, daß das Studium des für die Ernährung wichtigen pflanzenphysiologischen Aspekts der Beziehungen zwischen Aphiden und ihren Wirtspflanzen heutzutage wissenschaftlich und praktisch weit fruchtbarer sein dürfte als das Studium des auf die speziell pflanzlichen Sekundärstoffe bezogenen Aspekts, da bereits viel über die physiologische Variabilität bei den Pflanzen bekannt ist und da dies den Sammelpunkt aller landwirtschaftlichen Wissenschaft darstellt.

3. Zur Unterstützung dieser Auffassung wird über drei (weitgehend unveröffentlichte) Arbeiten über Aphiden (*Tuberolachnus salignus* Gmelin and *Aphis fabae* Scopoli) zusammenfassend berichtet. Sie zeigen, daß gewöhnliche Formen der physiologischen Verschiedenheit unter den Pflanzen, wie etwa Eigentümlichkeiten in Wachstum und Entwicklung oder im Wasserhaushalt, sowohl die Ernährung wie das Verhalten der Blattläusse beeinflussen. Mit der Zunahme weiterer Kenntnis solcher Unterschiede könnten wahrscheinlich im Hinblick auf die Aphidenresistenz von Pflanzen viel größere Fortschritte erzielt werden, als bisher durch Anbau- und Pflanzenzuchtmethoden für möglich gehalten wurde.

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DISCUSSION

A. J. THORSTEINSON suggested that the departure of aphids from leaves that have lost turgor might be due, in part, to a repellent high concentration of (for example) sugar.

J. S. KENNEDY agreed, mentioning some supporting experimental evidence, and pointing out that this would equivalent to the leaves becoming more mature.

Ent. exp. & appl. 1 (1958): 66—72. *North-Holland Publishing Co., Amsterdam*

THE BEHAVIOUR OF *APHIS FABAE* IN SELECTING ITS HOST PLANTS, ESPECIALLY DIFFERENT VARIETIES OF *VICIA FABA*

BY

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The different initial infestation of the two field bean varieties Rastatter and Schlanstedter (in the relation 1 : 3 till 1 : 5) by *Aphis* (*Doralis*) *fabae* Scop. is caused by a preference behaviour of the alates, which land on both varieties of bean in equal numbers, but whereas 99% leave Rastatter in a few minutes, only 90% leave Schlanstedter.

According to PAINTER (1951) the hereditary resistance of plants against infestation by insects may depend on three different ecological principles: Firstly on the tolerance of the host plant, that is not weakened or damaged by the infestation. Secondly on antibiosis, that is on the check of the growth or reproduction of the pest by an insufficient nutritive quality or by toxic effects caused by special properties of the plant or thirdly on preference, that is on individual choice of the insects, which prefer distinct species or varieties of plants more or less refusing others.

The fact, that the different varieties of beans are infested by the black aphids in a very different measure, has been known for a long time, but nearly nothing is known about the causes of these differences. No tolerance could be observed as yet. As the bean aphid must be looked upon as an extremely polyphageous insect, it seems improbable that the more or less distinct resistance of field bean varieties might be based on preference. DAVIDSON (1922, 1925) who, in the twenties, was the first to investigate the causes of the resistance of beans against aphids, therefore based his research entirely on an examination of the antibiotic effects of the plant. He placed a certain number of young adults on plants of the tested varieties and after a fixed time he counted the numbers of their offspring. As these numbers were strikingly different in the tested varieties, he considered that they gave a measure of resistance.

With a bean assortment at Quedlinburg, too, it was not possible to get a reproduceable series from resistant varieties to highly susceptible ones based on the reproduction rate. It appeared possible that there existed some mechanism of preference. In this we confined our investigations on the much susceptible variety Schlanstedter (S) and on the variety Rastatter (R) considered to be relatively resistant, thirty plants of either being cultivated repeatedly in an alternating double row of fifty to fifty centimetres. By a daily sampling of all alate aphids and the colonies of young larvae left by them respectively, we determined on every morning the mean infestation resulting during a period of 24 hours on each plant.

All repetitions yielded the difference of 1 : 3 to 1 : 5 between Rastatter and Schlanstedter. In the course of 147 test-days during the growing period in 1950, 695 initial infestations have been established on 60 Rastatter plants but 3427 on 60 Schlanstedter, that means 83% of the entire infestation being found on Schlanstedter. How constantly this primary settlement kept appearing is also made clear by the graph (fig. 1). Considering the frequency distribution on the days on which observation were made during the test years 1949 and 1950 of the height of the percentage infestation of both varieties, there is a maximum near 85% of S (sample mean 69,5 ± 29,1%). Obviously the winged aphids are able to distinguish the two varieties from one another and at the initial infestation they prefer the S 3 to 5 times more than the R. Considering the progressive reproduction of aphids it is evident that this different preference is the primary cause of the resistance observed by growers and farmers, even if an antibiotic effect could be proved as well.

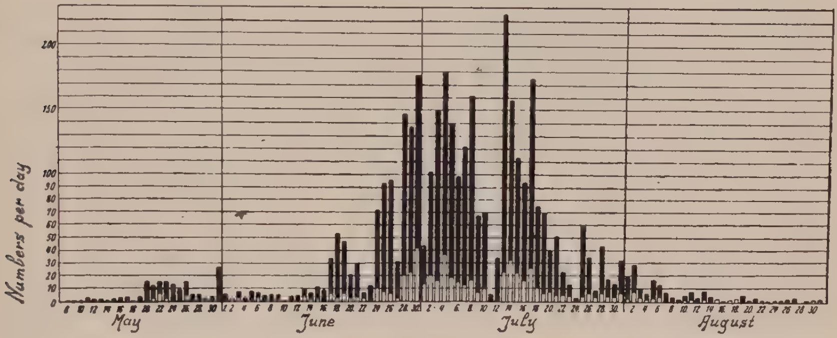

Fig. 1. The daily numbers of the initial infestations by *Aphis fabae* on 60 Rastatter (white) and 60 Schlanstedter (black) beans during the summer 1950

These results raise the question, how these differences of preference come about. Till now we have not succeeded in analysing the behaviour of the bean aphid in choosing its host plant in the laboratory or in the greenhouse by experiment, as it is hardly possible to keep winged aphids in the infestation stage. The young winged aphids are easy to breed, but they show a strong flying impulse connected with a distinct positive phototaxis and do not react either on host plants or on any stimulation to land. But while outdoors after a shorter or longer flight this phase turns over again to negative phototaxis and to a tendency to settle on host plants, the alates in the greenhouse will stay in the brightest place of the roof, starving rather than showing normal reactions to their host plants.

Therefore the further analysis of the preference resistance of *Vicia faba* to *Aphis fabae* could be tried only in the field, where the experimental conditions are not constant and the opportunities for observation are seldom favourable, but where instead of that the normal natural conditions prevail.

First of all we proved whether the preference applied to certain parts of the bean plant, or if all organs equally caused the difference in the initial infestation of the two varieties. It became evident in a series of tests with 30 decapitated R-

and S-plants each, that plants deprived of all vegetation points indeed showed a smaller total infestation, but the S — as in the control — kept showing 90% of the initial infestation. The same result came about when, reversely, all leaves were removed and only the sprouts were left intact. The suspicion that the extra-floral nectaries might cause the difference, was removed by experiments on plants without stipulae, for here also 80% of the initial infestation developed in S-plants. Even the reduction to one single primary leaf did not change the difference. There was the same result with cropped leaves freshened in water and arranged in the field in the usual mixed double row. Not matter whether primary or secondary leaves were used as a material, the S got about 80 to 83% of the daily found colonies.

Neither does the age of the leaves influence the difference in the preference of the two varieties. It is true: on tests on plants with 3 young, middle aged (ripe) and older (yellowish) leaves each, the total infestation decreased to 49, 33 and 18% respectively. But the S unvariably attracted 75 to 80% of initial infestations. Neither particular organs nor the physiological stage of the plants or leaves change the preference relation.

Before we know anything about the behaviour of the aphids infesting a new host plant it appeared possible that the initial infestation observed in 24-hour intervals was coming about by a primary choice, that is: at first arrival, at the first landing on a plant. One could believe it might be caused by the different smell of the varieties, so that the corresponding organs of the aphids allowed them to make a choice at a distance.

In the course of the last decade however some observations of KENNEDY (1950 a, b), and MOERICKE (1955) and the author (H. J. MÜLLER 1951 u. 1953) showed us that there is a great restlessness among the aphids in the infesting stage, and that of the numerous landing aphids only very few settle there definitely. The first hint in the direction of a secondary choice was brought about by the comparison of the initial infestation of the single plants with catches by yellow dishes that were observed simultaneously in a R- as well as in a S-plot at 24 hours' intervals. While the initial infestation was as usual with S, the number of *Aphis fabae* in the yellow dishes of both plots were practically always the same (fig. 2). Both plots had obviously been flown over by the same number of aphids in the infestation stage; however the S had been settled 5 times more frequently than the R. It remained still undecided whether the aphids had landed in equal numbers on both varieties and then most of them had left the R or if they had primarily avoided the latter.

Shortening the control intervals to 1 hour and finally to 20 minutes did not lead to any final proof. The percentage of the initial infestation on the S went down accordingly from 87 to 70 and 65% respectively, but this difference is not statistically significant.

All we could do was to catch uninterruptedly all the bean aphids alighting on two comparable plants of the two varieties over a period of many hours. During a period of more than 40 hours a total of 874 alate aphids were captured, 53,3% on R and 46,5% on S. The same plants, when exposed to colonisation by aphids both before and after these observations on alighting had been made showed the typical differences, i.e. 85% of the total infestations were on the S plant. This was definite proof that both varieties were primarily infested by equal numbers of infesting stage aphids and that the difference of preference exclusively arose from secondary choice, namely from "trying" or testing. This conception was confirmed by 27 hours' continued observation of, altogether, 600 alates alighted on two similar plants of both varieties during 9 different control periods in July 1952. Out of 312 alates alighted on R, only 3 definitely settled, that is: they bore some larvae (2 other cases remaining doubtful because of the nightfall), all but 1% left sooner or later. But on the S-plant 29 out of 289 alighting alates (+ 5 doubtful cases) remained for birth of larvae, that means 10%. It is therefore secondary choice that decides the difference of preference, but it is not simply that on the preferred variety all alighted aphids remain sitting and only from the less preferred plants the aphids fly away, but arrival and departure on both varieties are different in percentage. This significant difference of 1 : 10 of plants under constant observation is greater than at the daily counting of the initial colonies, where it is 1 : 5. This fact is clear if we consider that with the

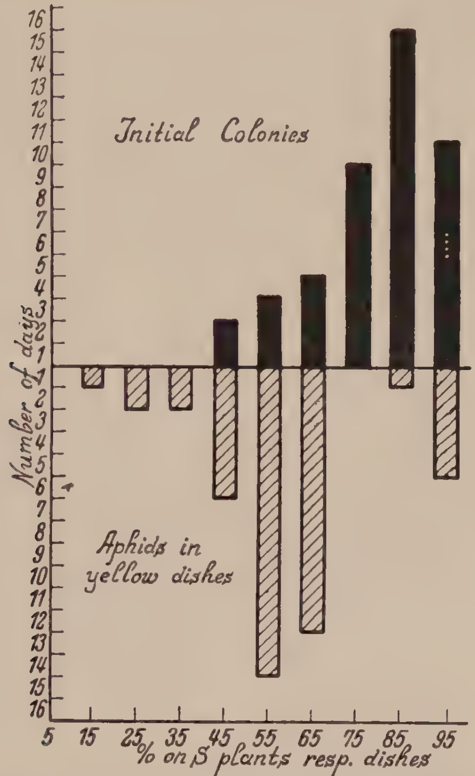

Fig. 2. Comparison between the daily numbers of the initial colonies on beans and the alate aphids (*Aphis fabae*) captured in yellow dishes in separated Rastatter (R) and Schlanstedter (S) plots (of 32 plants each) by the distribution of the control days according to their percentage of colonies on S-plants (black) respectively of specimens in the dishes of the S-plot (hatched).

latter method a great number of aphids are caught that have not yet definitely settled and that would soon have left. This amount however, being the same on both varieties, decreases the difference relation.

The fact that on the highly infestable and most preferred S bean only 10% of the alighted aphids remain sitting demonstrates that the choice of the host plant must be masked in a highly effective and variable manner by other factors or other ways of behaviour. The final settlement will always be smaller than the arrival. We know however that the intensity of the infestation flight is strongly dependent on the temperature, on the humidity and on the wind-speed. Accordingly the host preference is strongly dominated by two antagonistic reactions: the flying impulse and the infesting impulse, both of them being influenced contrarily by factors of environment. High temperature and humidity increase the flying impulse, lower ones increase settling. Low wind-speeds make possible unrestricted flying off and high wind-speeds may force an aphid to settle when it would have left the plant under calm conditions. A tired aphid on the contrary may settle under favourable flying conditions on a plant that it would have refused in a former phase.

It is very difficult therefore to tell anything definite about the real causes of the acceptance or refusal of one of the two varieties by the alighted aphids. The searching time of the future settlers, that is the time between the landing and the final settling, was with the 3 R-settlers 13, 14 and 46 minutes; with the 29 S-colonists it varies between 2 and 32 minutes with an accumulation between 2 and 17, especially between 2 and 10 minutes, the mean being 11 minutes. The mean staying time of visitors, that is the time between landing and taking off again, was on the contrary longer for those on S beans (6½ minutes) than for those on R beans (3½ minutes); these differences, notwithstanding the variability of the periods in different cases, are statistically significantly different. Therefore most of the visitors are leaving R-plants sooner than S-plants (fig. 3).

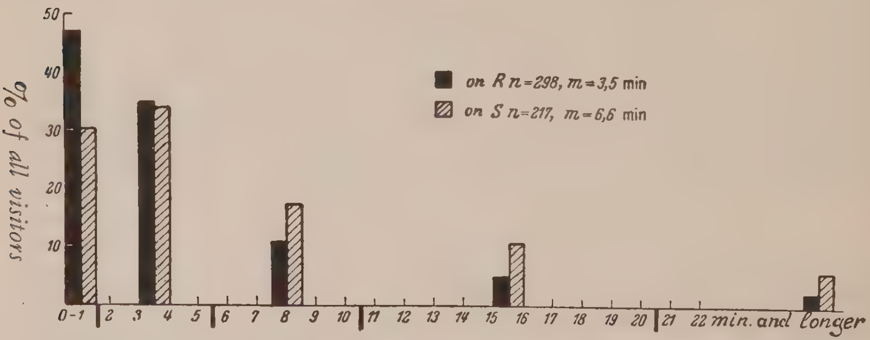

Fig. 3. Frequency distribution of the staying times of alatae (from alighting till take off) on a Rastatter and a Schlanstedter bean plant in percent of the total numbers of visitors during altogether 27 hours of flight.

During the searching and the staying time the alates wander about a great deal on the surface of the plant, however interrupting their wandering very often, setting up their proboscis and laying back their antennae. We could see by the remaining salivary sheaths that the so-called test feeding punctures (which mostly do not take more than 20 tot 40 seconds) very seldom penetrate beyond the epidermis and obviously penetrate into the intercellular tissue and constantly end blindly in the intercellular spaces. It seems improbable that on doing so they might suck up or taste anything.

The significance of the test feeding punctures is still unknown. As they end blindly it is doubtful whether the aphids imbibe by them any material for tasting. According to unfinished histological investigations the real feeding punctures pass always intercellularly to the phloem through tissues with no or little intercellular spaces. Therefore I should suppose the test feeding punctures to be a search for tissues having no intercellulars. These are normally found in young organs and in the surroundings of the vascular bundles, which are enveloped by sclerenchymatic or collenchymatic sheaths reaching to the epidermis. These are the very places, where aphids like to settle finally. Here the punctures automatically lead to the phloem. Probably the stylets are able to indicate by lever effects minute differences of pressure in the tissues. Therefore the aphid continues to insert its stylets only where it feels a close contact with the neighbouring cells. If we could prove this, we could suppose that the causes of the difference of preference were to be seen in the size and structure of the intercellular systems of the varieties. We could assume that the aphids succeed more often on susceptible varieties, less often on resistant ones to get to tissues with no or very few intercellular spaces, in order to find the phloem and so to be induced to settle down. In this way the statistical distribution of the infestation would be understandable.

Some of the aphids (alates and apterae) will change places once more or twice after the birth of the first or of a few young larvae. There is a distinctly perceivable tendency with the aphids to leave R-plants more frequently than S-plants, and at the next choice even the R-infestors prefer S-plants. Here perhaps an antibiotic effect is to be observed.

As we can now see the differences in resistance of the tested varieties of field beans arises first by a different host preference of the aphids. Moreover antibiotic effects may play a minor role too. The causes of the different preference are not clear as yet. It is sure only that the preference behaviour with varieties develops by a secondary choice not before the landing and is influenced by manifold exogenous factors (as plant condition, weather and so on) and by endogenous ones too (as age, physiological stage and motivation of the aphids).

ZUSAMMENFASSUNG

Als primäre Ursache des unterschiedlichen Befalls der beiden Ackerbohnensorten Rastatter und Schlanstedter durch die Schwarze Bohnenlaus, *Aphis (Doralis) fabae* Scop., wird Präferenzresistenz nachgewiesen. In mehrjährigen Beobachtungsreihen ergab sich bei 24-stündigen Kontrollen ein Unterschied in der Anzahl der Initialkolonien von 1 : 3 bis 1 : 5 auf Rastatter und Schlanstedter. Direktes Abfangen bzw. eingehende Beobachtung der auf den Pflanzen gelandeten Geflügelten zeigte, daß diese Initialbefallsdifferenz erst sekundär entsteht. Die Pflanzen beider Sorten werden statistisch in gleicher Häufigkeit beflogen, jedoch siedeln sich auf Rastatter nur 1%, auf Schlanstedter 10% der Gelandeten für längere Zeit (bis zum Absetzen wenigstens einer Junglarve) an. Die Bedeutung der sogenannten Probesaugstiche sowie der Größe und Verteilung der Interzellularräume in den Pflanzengeweben für das Auffinden des Phloems und somit für die Wirtswahl wird diskutiert.

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DISCUSSION

H. J. DE FLUITER: Wie tief versenken die Blattläuse beim „Probieren" ihre Stechborsten ins Pflanzengewebe, bevor sie sie wieder zurückziehen ?

H. J. MÜLLER: Die Probesaugstiche enden fast ausschließlich zwischen den Epidermiszellen bzw. in den Interzellularräumen der unmittelbar darunter liegenden Zellschicht, jedoch niemals in den Zellen selbst. Sie erreichen in keinem Falle ein Leitbündel.

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L'INFLUENCE DE LA PLANTE-HÔTE SUR LA FÉCONDITÉ DE L'INSECTE PHYTOPHAGE

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Pour étudier l'influence des constituants organiques de *Solanum tuberosum* sur la fécondité de *Leptinotarsa decemlineata* il faut d'abord tenir compte de la ration alimentaire d'entretien, et établir la relation entre fécondité et quantité d'aliment consommé.

Les variations du rapport C/N sont moins importantes que celles de la teneur en lipides choliniques sur la fécondité du Doryphore; cependant un supplément de caséine augmente le taux de ponte si la feuille est pauvre en azote. Le glucose ralentit l'activité ovogénétique mais son effet est plus difficile à interpréter car il joue un rôle énergétique important dans l'activité musculaire.

L'effet des lipides phosphatidiques est plus nettement mis en évidence dans les tests biologiques et dans les tests biochimiques.

Les facteurs alimentaires de la fécondité ont été moins étudiés que ceux de la croissance et ces derniers sont moins bien connus chez les Insectes phyllophages que chez les espèces dont le régime alimentaire peut être artificiellement constitué (Diptères saprophages et hématophages, Coléoptères et Lépidoptères détritiphages ou granivores); ce que l'on constate en consultant les revues générales récentes de W. TRAGER (1953) et de Z. H. LEVINSON (1955).

L'influence du régime alimentaire, caractérisé par l'espèce botanique de la plante-hôte, sur la ponte est toujours marquée chez les Insectes oligophages mais n'est pas négligeable chez les polyphages. TAUBER et al. (1944) établissent une variation de fécondité de 1 à 7 chez les femelles de *Melanoplus bivittatus* Say nourries sur 29 végétaux différents; l'indice de fécondité (ou "quantité moyenne d'oeufs pondus par femelle et par jour") est le suivant pour quelques plantes:

Lycopersicon esculentum	1.0
Alopecurus pratensis	1.8
Cucurbita pepo	2.6
Cannabis sativa	3.3
Melilotus sp.	3.9
Trifolium sativa	4.9
Medicago sativa	6.0
Lactuca sp.	6.9

Sur un autre Acridien, *Melanoplus mexicanus mexicanus* Sauss., O. L. BARNES (1955) étudie l'influence de quelques plantes-hôtes fondamentales; il conclut que la Luzerne est moins favorable qu'on ne le pensait pour le développement et la reproduction de cet Insecte, et il donne comme quantité d'oeufs par femelle nourrie zur Luzerne (*Medicago sativa*): 13, sur *Chenopodium murale*: 43, sur „Johnson grass": 89 et sur *Sisymbrium irio*: 196.

Chez *Leptinotarsa decemlineata* Say, Trouvelot et Grison (1935) ont montré l'influence de diverses espèces d'un même genre botanique; le nombre total de pontes déposées par 6 couples répartis en trois cages est :

— sur *Solanum edinense* Berth. = 35 pontes comptant en moy. 35 oeufs par ponte
— sur *S. tuberosum* L. = 29 — — — — 25 — — —
— sur *S. utile-demissum* Lindl. = 13 — — — — 12 — — —
— sur *S. Jamesii* Torr. = 8 — — — — 8 — — —
— sur *S. Commersonii* Dun. = 0 —

Enfin Dahms et al (1936) constatèrent des phénomènes analogues chez *Blissus leucopterus* Say vivant en présence de variétés différentes de Sorgho:

— sur la var. „Dwarf Yellow milo" la ponte moy.par jour est de 3.5 oeufs
— — — „Blackhull kafir" — — — — — — 1.3 —
— — — „Atlas sorgo" — — — — — — 0.4 —
— — — „Common feterita" — — — — — — 0.2 —

Bien entendu, selon le type d'évolution des gonades, il y a lieu de distinguer entre l'influence du régime alimentaire de l'imago et l'influence de la nutrition larvaire sur l'ovogénèse et la fécondité.

Ce dernier cas est celui du Coléoptère *Bruchus obtectus* Say dont les femelles n'exigent pas, dans les conditions normales, une alimentation spéciale pour pondre (G. M. Herford, 1935), bien que, chez *Bruchus quadrimaculatus* Fab., A. O. Larson (1924) ait constaté un accroissement de fécondité en offrant expérimentalement aux femelles de l'eau sucrée (33 oeufs par femelle nourrie contre 23 oeufs par femelle jeûnant). Il en est de même chez *Pectinophora gossypiella* Saund. (Lukefahr, 1956).

Le fait est assez fréquent chez les Lépidoptères; chez *Lymantria monacha* L. la fécondité croît en fonction du poids des nymphes résultant d'une alimentation larvaire variable : avec le Sapin elle est de 0 à 137 oeufs et avec le Chêne de 178 à 374 oeufs (H. Sattler 1939).

Nous avons étudié plus particulièrement l'action de l'état physiologique de la plante-hôte *Solanum tuberosum* L. et celle de certains constituants biochimiques du feuillage, c'est-à-dire l'influence des facteurs alimentaires intrinsèques, sur la fécondité du Doryphore, *Leptinotarsa decemlineata* Say.

Ces études ont été précédées par l'analyse de l'influence des facteurs externes sur la fécondité de l'Insecte, qui a été publiée ailleurs (Grison, 1948, 1957), mais dont il faut tenir compte dans l'interprétation des résultats obtenus en expérimentant sur les constituants alimentaires.

METHODE EXPERIMENTALE ET RATIONS ALIMENTAIRES

Les méthodes et techniques d'essais que nous avons utilisées font l'objet d'une description détaillée ailleurs (1957). Il est fondamental de rappeler que la vie imaginale du Doryphore comporte ordinairement trois phases caractérisées par des besoins physiologiques, des états éthologiques et des exigences écologiques différents; ce sont: la phase de maturation de courte durée au cours de laquelle l'insecte parfait achève, après la mue imaginale, le développement de ses gonades; la période de diapause qui peut ne pas se manifester dans certaines circonstances (GRISON, 1944a; DE WILDE, 1954); et la phase de reproduction qui se prolonge d'avril en août sous le climat parisien.

Tous les animaux d'expériences rapportées ici ont été prélevés au début de la phase reproductrice; ils étaient placés par couples séparés, ou le plus souvent même les femelles étaient isolées après accouplement, sous des petites bonnettes de toile métallique parfaitement aérées.

La ponte et la consommation quotidiennes de chaque femelle étaient évaluées au cours des essais dont la durée était le plus souvent limitée à des périodes de „test biologique" de 10 à 20 jours après une période de contrôle préalable de la fécondité. La fécondité est généralement exprimée par un „indice de fécondité" représentant la ponte moyenne par femelle et par jour. La signification de cet indice est discutée ailleurs; il y a une corrélation hautement significative entre la quantité d'oeufs et le nombre de pontes; en outre la tendance présentée par une femelle d'être plus ou moins féconde se maintient pendant toute sa vie, en sorte qu'il y a intérêt à établir, dans les tests biologiques, la comparaison entre deux traitements dont un sert d'étalon avec les individus d'un même lot.

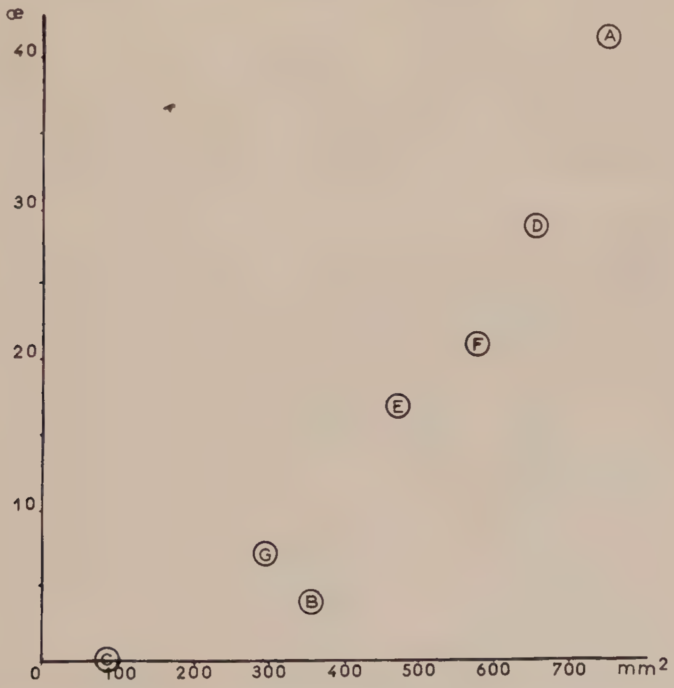

Fig. 1. Relation entre la fécondité moyenne (en oeufs) et la quantité d'aliment consommé (en mm² de surface foliaire) chez *L. decemlineata*, par individu et par jour.

Nous établissons d'une manière analogue un „indice de consommation" pour chaque femelle représentant approximativement la quantité moyenne d'aliment consommé par individu et par jour et exprimée en surface foliaire. Dans les expériences de base cette évaluation a été faite d'une manière rigoureuse.

Nous reproduisons à nouveau le graphique montrant la corrélation élevée entre fécondité et consommation (fig. 1). Cependant, si l'on fait porter l'analyse sur six périodes hebdomadaires successives, l'analyse de covariance indique que la fécondité par période reste significative après avoir tenu compte de la consommation tandis que la fécondité par individu dans les mêmes conditions devient non significative. Ceci traduit le fait que, pendant les dernières semaines, la consommation est tombée au-dessous d'un „seuil quantitatif'' assurant l'ovogénèse et la ponte ou encore que les besoins alimentaires sont réduits simultanément avec la diminution du potentiel reproducteur.

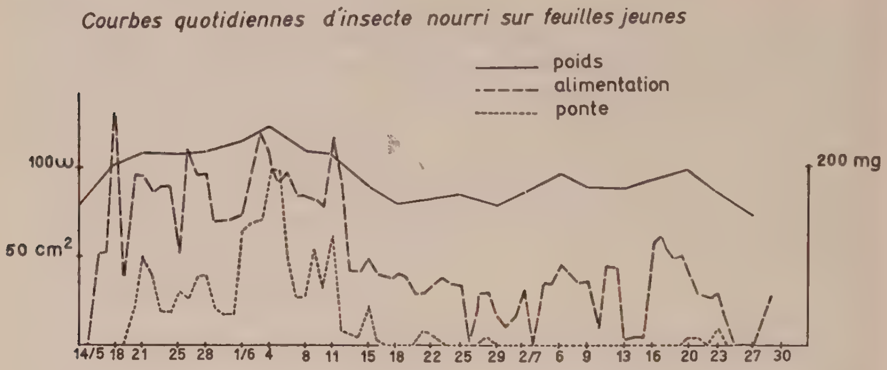

Fig. 2. Courbes quotidiennes de consommation, de ponte et courbe bi-hebdomadaire de poids d'une femelle de *L. decemlineata* nourrie sur feuilles jeunes de Pomme de terre.

Ce sont deux aspects d'un même problème que nous étudierons plus tard mais que schématisent les graphiques des fig. 2 et 3; ils sont empruntés à une expérience dans laquelle l'évolution pondérale des individus a été suivie à raison de deux pesées par semaine et parallèllement à l'observation quotidienne rigoureuse de la ponte et de la consommation: la fig. 2 en donne un exemple pour l'une des dix femelles de l'essai. Elle met très bien en évidence les faits suivants :

1. la phase reproductrice comprend deux périodes distinctes : l'une d'activité ovogénétique pendant laquelle les besoins énergétiques correspondant à cette fonction physiologique se traduisent à la fois par une consommation élevée et un accroissement appréciable du poids de l'insecte; l'autre de sénescence ou d'épuisement ovogénétique au cours de laquelle le poids de l'animal se maintient un peu au-dessus du poids initial jusqu'à une brusque diminution ante-mortem correspondant à la sénescence organique totale (Grison et Le Berre, 1953);
2. la consommation durant cette deuxième période représente approximativement la ration alimentaire d'entretien y compris les besoins énergétiques requis par une activité musculaire modérée;
3. le seuil quantitatif de consommation, établi dans les expériences interprétées par le graphique de la fig. 1, devrait correspondre à cette ration d'entretien.

Nous avons alors établi, pour 4 femelles ayant vécu et pondu assez régulièrement au moins pendant deux mois, les courbes de rendement ovogénétique de la fig. 3. E. Titschack (1924) est, je pense, le premier auteur à avoir suggéré cette notion: chez *Carausius morosus* Br. & Redt. il établit que pour produire 1 mg. d'oeuf, l'Insecte doit consommer 7,8 à 10,4 mg. de nourriture. Pour nous, cet indice correspond au rapport de la quantité d'oeufs pondus entre deux observations à la quantité d'aliment consommé, en cm² de feuillage, pendant le même temps. Il devrait être corrigé, ainsi que l'a fait LAFON (1950) chez le *Carausius*, en tenant compte de la ration d'entretien et en fonction du poids de l'animal par une analyse de covariance qui sera faite ailleurs, c'est-à-dire en établissant la „ration alimentaire de croissance ovulaire".

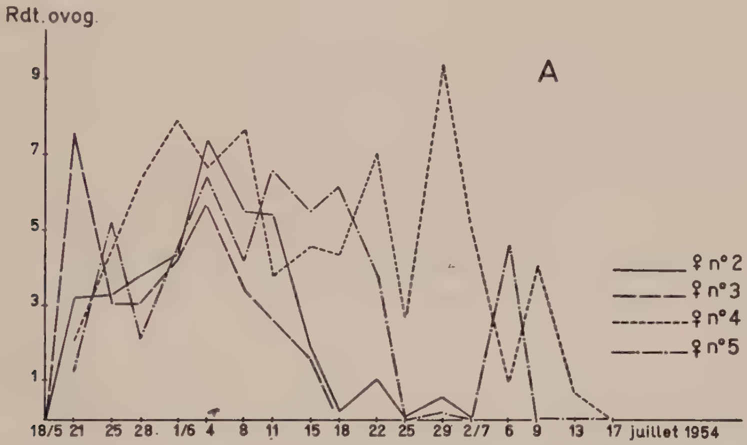

Fig. 3. „Rendements ovogénétiques" de 4 femelles de *L. decemlineata* au cours de leur phase reproductrice.

Néanmoins cet élément brut nous indique que le potentiel d'activité reproductrice, correspondant à des échanges métaboliques intenses, est maximum pendant une période de durée variable selon les individus; le rendement ovogénétique brut est en moyenne, pour les 4 femelles considérées dans le graphique de la fig. 3, au cours des huit semaines successives de : 3,8; — 4,0; — 5,8; — 4,5; — 4,3; — 3,8; — 1,8; — 0,9 oeufs par cm² d'aliment.

Il y a donc intérêt, du point de vue méthodologique, à réduire la durée des tests biologiques de fécondité et à prélever les femelles pendant la même période d'activité imaginale. Dans ces conditions le rendement ovogénétique brut peut être utilisé comme élément d'appréciation et de comparaison de la valeur de régimes alimentaires qualitativement différents, car il tient compte de la variation quantitative de la ration. Malheureusement les difficultés d'évaluation pondérale rigoureuse de cette dernière qui nous ont amené à utiliser de préférence l'estimation de la surface foliaire consommée, limitent la portée et l'utilisation du rendement ovogénétique.

Enfin il ne faut pas négliger de mentionner les différents processus sensoriels et humoraux grâce auxquels, sous l'influence de la croissance des gonades et de la multiplication des cellules germinales, l'organisme répond à des besoins énergétiques accrus par une augmentation de consommation: „la ponte stimule fortement l'appétit" (LAFON, 1951).

Ce stimulus peut être d'origine endocrine: JOHANSSON (1954) a obtenu le déclanchement de la ponte chez *Oncopeltus fasciatus* Dallas par la transplantation dans les femelles immatures et à jeun, de corpus allatum normalement développés provenant de femelles alimentées. Il dépend aussi de mécanismes sensoriels dont DETHIER (1953, 1954) a étudié en détail le fonctionnement. Mais nous ne prendrons pas en considération dans ce travail les facteurs du comportement qui participent à la fois au rationnement quantitatif et à la sélection alimentaire, ce qu'a parfaitement analysé DE WILDE (1958) chez la larve de *L. decemlineata.*

VARIATION DU CHIMISME FOLIAIRE CHEZ SOLANUM TUBEROSUM

La plante subit des modifications biochimiques au cours de son développement et aussi selon les conditions de nutrition du végétal.

Les données analytiques fournies par la physiologie végétale ne sont pas négligeables lorsqu'on étudie les effets de la qualité de l'aliment offert aux Insectes sur la fécondité des femelles (TRAGER, 1941; WIGGLESWORTH, 1950); mais elles ne peuvent nous renseigner utilement sur l'efficacité d'un élément biochimique qu'à la condition de pouvoir établir des corrélations statistiquement significatives entre ces données analytiques et les résultats des tests biologiques de fécondité.

L'examen du rapport C/N a d'abord été pris en considération pour deux raisons: d'abord parce que les entomologistes ont attribué un rôle tantôt aux glucides, tantôt aux matières azotées dans la fécondité des insectes; ensuite parce que ce rapport, depuis KLEBS (1918), a pris une grande importance en physiologie végétale et qu'il est caractéristique de l'évolution du chimisme de la plante au cours de son développement.

Les conditions de culture sont susceptibles de modifier la teneur en azote total et en glucides du feuillage d'une plante et par conséquent de modifier le rapport C/N; c'est ainsi que le chimisme de végétaux, tels que des Crucifères (EVANS, 1938) ou des Légumineuses (BARKER, 1952; BARKER et TAUBER, 1954) a été modifié en relation avec l'étude de la reproduction chez les Aphides. Elles ne présentent probablement pas le même intérêt pour les Insectes broyeurs et les Insectes piqueurs.

TABLE I

Conditions de culture en 1953 (3ème expér.)	Sucres[1]) réducteurs	Sucres totaux	Azote total	C/N	Indice fécondité
terreau ombré	6,1	16,5	67	0,25	9,1 oeufs
terreau ensoleillé	16,8	47,0	58	0,81	10,8 —
limon ensoleillé	13,3	28,5	94	0,31	7,1 —
limon ombré	9,0	14,2	74	0,19	6,1 —

[1]) en p.p. 1000 du poids sec de feuilles de la var. „Bintje".

Les différences de fécondité que nous avons obtenues avec le Doryphore nourri sur des feuilles de Pommes de terre développées soit en terreau, soit en limon, soit sous ombrage, soit en plein soleil, n'ont pas été statistiquement significatives (Table I).

Il ne faut pas perdre de vue que les conditions de nutrition et de croissance des végétaux, sont affectées par les variations annuelles des facteurs climatiques, en sorte que celles-ci peuvent influencer indirectement le développement et la fécondité des Insectes phytophages par leurs effets sur les plantes-hôtes (UVAROV, 1931).

D'autre part, la composition chimique des feuilles est constamment modifiée au cours de chaque jour sous l'effet de la photosynthèse: à la fin de la journée, le feuillage est plus riche en sucres réducteurs qu'au lever du jour, tandis que la teneur en azote varie peu, en sorte que le rapport C/N s'en trouve assez considérablement modifié.

Des rameaux prélevés à des heures différentes n'ont donc pas la même composition chimique et peuvent être qualitativement différents pour les Insectes qui s'en nourrissent. DE JONG (1938) a montré ainsi que certains *Epilachna* pondent davantage lorsqu'ils sont nourris de feuilles de Thé cueillies le soir et riches en glucides, tandis que *Helopeltis theivora* Waterh. ne réagit pas de la même manière.

Nous avons opéré de même avec *L. decemlineata* après avoir constaté que les modifications chimiques étaient négligeables au cours de la journée dans les feuilles de Pomme de terre séparées des plantes et mises à tremper dans le petit tube d'eau de la cage d'essai. Il n'est pas nécessaire de reproduire les résultats peu significatifs publiés ailleurs (GRISON, 1952; 1957) et dont certains figureront plus loin à d'autres occasions.

Enfin, parallèlement aux études entreprises par COIC (1945) sur les variations de la constitution organique et minérale du feuillage de Pomme de terre au cours de la croissance du végétal, nous avons recherché l'influence de celle-ci sur la fécondité du Doryphore et constaté que la ponte est inhibée chez les femelles nourries avec du feuillage sénescent ou des feuilles de plantes âgées (GRISON, 1944b; 1947b).

F. PICARD (1926) avait déjà supposé que la sénescence des tissus foliaires constituait pour *Haltica ampelophaga* Guer une cause de diminution de fécondité.

LEGAY et Melle BAUD (1953) et LEGAY (1954) ont montré un phénomène comparable chez *Bombyx mori* mais, dans le cas de la plupart des Lépidoptères, c'est la nature de l'alimentation larvaire qui retentit sur la vitalité et la fécondité de l'imago: il en est ainsi chez le Bombyx Cul-brun, *Euproctis chrysorrhoea* Don. (GRISON, 1956) dont les femelles issues de larves nourries de feuilles sénescentes de Pommier depuis le troisième stade déposent des pontes de 100 à 200 oeufs tandis que celles provenant de chenilles avec régime de feuilles jeunes pondent chacune 350 à 400 oeufs.

BLAIS (1953) a constaté une relation analogue chez une Tordeuse, *Choristoneura fumiferana* Clem., dont la fécondité est réduite au moins de moitié sinon du tiers si les femelles sont nourries de feuillage d'Epicea de l'année précédente au lieu de s'alimenter aux dépens de celui de l'année.

En étudiant plus particulièrement l'apparition des différentes formes d'Aphides, KENNEDY et BOOTH (1951, 1954) ont étudié la signification physiologique et écologique de l'âge de la feuille sur le processus de la succession des formes.

En ce qui concerne le Doryphore, nous avons déjà précisé que la valeur qualitative de l'aliment a un effet immédiat sur la fécondité de l'Insecte (GRISON, 1952) et que la feuille jeune a des effets presque semblables quel que soit l'âge du végétal sur lequel elle a été prélevée (Grison, 1957).

Nous nous sommes alors attaché à caractériser les différences biochimiques pouvant exister entre feuilles jeunes et feuilles vieilles de *Solanum tuberosum*. Des résultats analytiques ont été publiés en ce qui concerne l'azote total, les glucides et la choline totale; mais nous en reproduisons les caractéristiques essentiels en un tableau dans lequel sont portés également les indices de fécondité des Insectes nourris avec du feuillage analogue à celui qui a été analysé (Table II).

Table II

Aliment : Ackersegen	Feuilles âgées		Feuilles jeunes	
Prélévement à	9 h.	17 h.	9 h.	17 h.
en 1949				
sucres totaux	44,0	55,7	55,6	63,6
azote total	43,0	55,0	41,0	47,0
C/N	0,63	0,83	1,10	1,16
indice fécondité	*13,3 oeufs*	*3,6 oeufs*	*2,2 oeufs*	*2,3 oeufs*
en 1953				
sucres totaux	32,0	50,0	25,0	42,6
azote total	61,0	69,0	28,0	33,5
C/N	0,52	0,72	0,89	1,27
indice fécondité	*11 oeufs*	*20 oeufs*	*2 oeufs*	*1 oeuf*
en 1954				
indice fécondité	*26,7 oeufs*	*22,6 oeufs*	*7,8 oeufs*	*8,6 oeufs*
choline totale	0.274		0,079	

Il ressort de ces données que la choline totale symbolise le mieux la différence d'indice de fécondité: cette substance a retenu plus particulièrement notre attention. Les données analytiques nombreuses qui ont été recueillies (Ducet, Kahane et Grison, 1957) mettent en évidence:

a) — que la teneur en substances choliniques des tissus végétaux et notamment du parenchyme foliaire de *Solanum tuberosum* diminue progressivement au cours de la maturation et du vieillissement de ceux-ci ;

b) — que la choline, sous toutes ses formes, est constamment en plus grande quantité chez les femelles que chez les mâles de *Leptinotarsa decemlineata* ; ensuite, qu'elle subit des variations très importantes au cours de l'évolution de l'Insecte (de 0,535 à 1,540 mg de choline totale par gr. de poids frais de l'animal) et que c'est en période reproductrice que l'animal est le plus riche en choline totale.

RELATIONS ENTRE LA TENEUR DE L'ALIMENT EN CONSTITUANTS ORGANIQUES ET LA FECONDITE DU DORYPHORE

L'utilisation, par l'organisme du Doryphore, des groupes de substances organiques normalement présentes dans la feuille de Pomme de terre a été contrôlée sans étude systématique; ainsi le „coefficient de rétention azotée" a été évalué à 50%; pour les lipides choliniques la rétention est un peu supérieure à la moyenne (53%) chez les femelles après l'émergence printanière, tandis qu'elle est presque totale après la mue imaginale ce qui caractérise bien le rôle d'"éléments constants" donné par Terroine (1919) à ces substances.

Pour apprécier, dans les tests biologiques, l'effet d'un constituant sur la fécondité nous avons eu recours à deux procédés essentiels : soit d'offrir des feuilles physiologiquement différentes et caractérisées par l'analyse chimique faite simultanément sur des échantillons de plante prélevés à cet usage; soit d'offrir des feuillages aussi identiques que possible par leur origine et leur état physiologique mais recouvert d'une substance chimique définie : c'est la „supplémentation" de l'aliment naturel.

Nous commenterons quelques expériences inédites de supplémentation pour justifier les conclusions que nous avons émises (Grison, 1957) dans un travail où sont rassemblés la plupart des essais et tests éxécutés pendant douze ans[1]).

Constituants azotés et glucidiques

La plupart des expériences reproduites ailleurs montrent des résultats non significatifs, notamment si l'on considère les cas où les suppléments biochimiques sont apportés à des feuilles jeunes. Le graphique de la fig. 4 schématise cette interprétation.

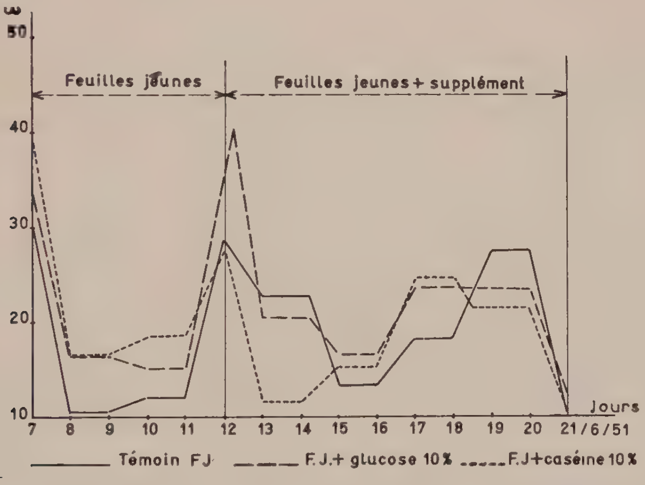

Fig. 4. Fécondité de trois lots de femelles de *L. decemlineata* pendant deux périodes successives avec et sans „suppléments".

Cependant, lorsqu'on supplémente des feuilles vieilles prélevées le matin ou le soir avec de la caséine on obtient une augmentation significative de la fécondité des Doryphores soumis à l'essai (Table III).

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[1]) En douze ans, à la Station Centrale de Zoologie agricole de Versailles et pour la seule étude des facteurs alimentaires qualitatifs, notre Laboratoire a observé individuellement et quotidiennement la ponte et la consommation de plus de 4.600 femelles de Doryphores groupées en 510 lots ou séries d'aliments distincts et définis dont 85 étaient suivis pendant plusieurs périodes et parfois même pendant toute la vie imaginale avec pésée plus ou moins fréquente des Insectes.

Dans l'expérience 1953, l'unité de surface d'un cm^2 de feuille âgée pesant approximativement 30 mg., représente une teneur moyenne de 0,9 mg. d'azote total. Le supplément caséine étant d'environ 0,7 mg. par cm^2, l'unité de surface de feuille supplémentée représente alors une teneur de 53 pp. 1000 d'azote, voisine de celle de la feuille jeune.

Table III

Exp.	A. Régimes avec feuilles seules				B. avec caséine		Rapport indices B/A
	Aliment support (Ackers.)	Teneur azote pp. 1000	Ponte totale oeufs	Indice fécond. oeufs	Ponte totale oeufs	Indice fécond. oeufs	
1953	F. jeune 9h	61,0	715	11,1	478	7,5	0,7
	F. — 17h	69,0	1617	20,0	1688	26,3	1,3
	F. âgée 9h	28,0	128	2,0	527	8,2	4,1
	F. — 17h	33,5	61	1,0	288	4,2	4,2
1954	F. jeune 9h	—	2673	26,7	3091	33,1	1,2
	F. âgée 9h	—	781	7,8	1022	11,7	1,5
	F. — 17h	—	859	8,6	2082	20,8	2,4

Aussi peut-on admettre que l'apport d'un supplément azoté à la ration accroît la fécondité du Doryphore lorsque la teneur de l'aliment naturel (ici la feuille âgée de variété "Ackersegen") en azote est faible.

Dans le cas de supplémentation de feuilles jeunes naturellement riches en azote total, tout se passe comme si l'Insecte manifestait un besoin quantitatif minimum d'azote, et qu'il était susceptible de réduire son taux de consommation jusqu'à la satisfaction nécessaire et suffisante de ce besoin.

Ainsi dix individus consomment en 48 h. 52,6 cm^2 de feuilles normales pendant que la consommation de dix autres individus est réduite à 43,7 cm^2 lorsque les feuilles sont supplémentées en caséine; mais la quantité d'azote ingérée est cependant plus forte dans ce deuxième lot (10,49 mg. d'azote) que dans le premier lot (8,31 mg. d'azote).

Il nous a été difficile de prendre en considération les formes qualitatives de l'azote; au cours des quelques essais tentés avec des régimes d'acides aminés variés, nous avons envisagé l'hypothèse que certains d'entre eux (ou leur mauvaise proportion) seraient non-appétitifs; en effet dans un lot nourri avec des feuilles jeunes supplémentées par un mélange d'asparagine, d'alanine et de tyrosine, la consommation était réduite au tiers de la normale, ce qui est considérable.

En ce qui concerne les glucides, certains essais montrent, inversement au cas de l'azote, qu'un supplément provoquerait une diminution de ponte, ou plus vraisemblablement un ralentissement de l'ovogénèse (voir fig. 6, évolution morphologique des ovarioles), lorsque l'aliment en serait naturellement riche et qu'il y aurait „surcharge" de glucides.

Ainsi le rapport des indices de fécondité entre deux périodes dont la première comporte la feuille jeune seule et la seconde un supplément est de:

$\frac{12{,}6}{4{,}3} =$ 2,9 fois plus faible avec saccharose

$\frac{17{,}4}{6{,}7} =$ 2,6 — — avec glucose

$\frac{15{,}3}{9{,}7} =$ 1,6 — avec glucose + lécithine

$\frac{11{,}1}{9{,}1} =$ 1,2 — avec feuille témoin.

Ce qui semble signifier que, malgré une diminution de fécondité au cours de la seconde période, cette diminution est becaucoup plus forte avec un supplément glucidique mais qu'elle redevient comparable au témoin si l'on ajoute également un supplément lécithinique.

Néanmoins la diversité des résultats obtenus nous oblige à une conclusion prudente sur le rôle modérateur des glucides; nous pensons, qu'en raison de leur destinée et de leur plasticité métaboliques, l'effet indirect des glucides alimentaires sur la fécondité du Doryphore dépend essentiellement de l'intensité des besoins énergétiques de l'Insecte.

Constituants phospholipidiques

De nombreuses expériences ont montré l'action inhibitrice de feuilles vieilles ou étiolées sur la ponte de *L. decemlineata.*

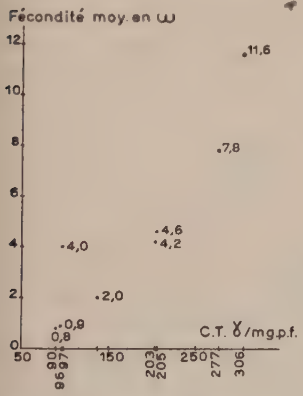

Fig. 5. Relation entre la fécondité moyenne (en oeufs) de *L. decemlineata* et la richesse de l'aliment en choline totale (par mg. de feuille fraîche).

La corrélation la plus significative entre richesse de l'aliment en lipides choliniques et fécondité du Doryphore a été établie à l'occasion d'une étude comparative des qualités nutritives du feuillage de diverses variétés de Pomme de terre (fig. 5).

Par la méthode de supplémentation et au cours de nombreuses expériences, tantôt très significatives et tantôt beaucoup moins, nous confirmons les données de cette corrélation en constatant la tendance très fréquente à l'accroissement de fécondité des femelles alimentées avec du feuillage recouvert d'une émulsion de lécithines[1]).

Nous venons de citer l'effet d'un supplément lécithinique ajouté au supplément glucidique. Dans la même expérience, des lots de femelles nourries sur feuilles âgées („Bintje") pendant une première période de 8 jours, puis de feuilles analogues supplémentées avec diverses émulsions de lécithines pendant les 8 jours suivants, présentent cette tendance à l'acroissement de fécondité comme l'indiquent les rendements ovogénétiques ci-dessous, après correction approchée pour tenir compte de l'augmentation de poids sec due au supplément (Table IV).

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[1]) Nous avons utilisé dans les expériences relatées ici des lécithines d'oeufs du commerce; elles sont mises en émulsion dans un mortier en versant d'abord une goutte d'alcool 70° puis en ajoutant goutte à goutte l'eau ou une solution physiologique en quantité suffisante (MAYER et TERROINE, 1907); lorsque l'émulsion est préparée au mixeur il peut se produire une destruction de certains constituants thermolabiles (GRISON, 1957). Une émulsion à 2 p. 100 de lécithines épandue au pulvérisateur Richardson sur une feuille vieille d'Ackersegen permet d'obtenir un aliment dont la teneur en choline totale pp. 1000 est voisine de 0,400.

Table IV

1ère période		*2ème période*	
avec f. âgée seule	4.5 oe/cm^2	sans supplément	3.5 oe/cm^2
—	5.3 —	avec lécithines 2% (1)	5.8 —
—	4.1 —	avec léc. 2% et MgCl$_2$	5.5 —
—	4.6 —	avec lécithines 8%	5.9 —

Le plus souvent l'indice de fécondité obtenu avec l'alimentation „feuille âgée supplémentée" reste inférieur à celui obtenu sur „feuille jeune", malgré son accroissement par rapport à l'indice sur „feuille âgée seule". En outre un supplément de lécithines sur feuille jeune est rarement efficace.

Le phénomène est mis en évidence avec des lots de 10 femelles nourries d'une manière semblable pendant 6 jours avec des feuilles âgées de variété „Ackersegen" pris pendant une deuxième période de 15 jours avec des aliments différents (Table V).

Table V

1ère période		*2ème période*		
ponte totale	rend.ovog.	aliment	ponte totale	rend.ovog.
602 oe.	20 oe/cm^2	f.jeune	2500 oe.	67 oe/cm^2
532 —	18 —	f.âgée	354 —	31 —
681 —	22 —	— + lécith. 0,5%	775 —	46 —
330 —	18 —	— + lécith. 2,0%	230 —	46 —

Le taux d'accroissement entre les deux périodes obtenu sur f. âgée supplémentée en lécithines (2 et 2,5) est un peu supérieur à celui obtenu avec la même feuille, sans supplément (1,5) mais il est inférieur à celui de la feuille jeune (3,5).

Cela peut être dû: soit au fait que le supplément lécithinique ne correspond pas qualitativement à la ration phosphatidique recherchée par l'organisme de l'Insecte, soit à un effet de masquage de l'action des lipides lécithiniques dû à la présence dans la feuille vieille d'éléments freinateurs tels que certains glucides.

Mais on peut également évoquer l'absence d'oligo-éléments organiques ou minéraux, en particulier des phosphates dont la feuille s'appauvrit au fur et à mesure qu'elle approche de sa maturité (R. Fraisse, 1952). Une indication, malheureusement insuffisante, sur le rôle des phosphates nous est donnée dans une seule expérience d'une durée de 22 jours mais avec 5 femelles seulement par lot dont l'aliment était constitué par :

A. une émulsion à 5% de lécithines dans l'eau distillée
B. — — dans un glycérolé à 10% de glycérol
C. — — dans un glycérolé à 10% et contenant 3% de phosphate de magnésium et 2% de phosphate bicalcique

Pour établir le rendement ovogénétique, on a corrigé la surface foliaire par un équivalent en poids du supplément:

avec feuille jeune, ponte totale = 606 oeufs; rend.ovog. = 15 oe/cm^2
avec f.j. et suppl. A, — = 423 — ; — = 16 —
— — B, — = 394 — ; — = 13 —
— — C, — = 715 — ; — = 45 —

Avec un support feuille âgée la ponte est nulle dans le témoin et très faible dans les essais (rendement de 0,5 oeuf); cependant avec le supplément phosphaté „C” la fécondité est 6 fois plus forte qu'avec les suppléments „A” et „B”.

Des émulsions de lécithines à 5% enrichies en chlorhydrate de choline aux doses de 0,1 et 0,2 p. 100 ont été efficaces dans une expérience dont les témoins étaient malheureusement médiocres.

Mais la deuxième émulsion de lécithines-choline incorporée à une poudre de feuilles jeunes de „Bintje” desséchée à 100° C. provoque une reprise de la ponte, lorsque celle-ci a été interrompue pendant un mois par une sous-alimentation sur un aliment peu appétitif constitué par un support normal recouvert seulement de la même poudre de feuilles; nous donnons les fécondités individuelles pendant les périodes successives pour caractériser le phénomène (Table VI).

TABLE VI

périodes	aliment	fécondités individuelles						féc. moy.par fem.
15—24 mai 1948	témoin	109	131	134	171	206	124	146 oeufs
27 mai—18 juin	avec poudre	0	22	42	15	10	10	16 —
19 — 28 juin	d°	0	0	17	0	0	0	3 —
29 juin—21 juil	avec suppl.	173	15	25	42	59	0	52 —

Les poudres de feuilles desséchées de Pomme de terre avaient été préparées pour la mise au point de régimes artificiels (GRISON, 1947a); elles ont été ajoutées avec une charge de bentonite sur des feuilles de Pois, comme support neutre, dans certaines expériences qui ont confirmé l'indication précédente.

Ces expériences, citées ailleurs, font apparaître d'une part que la feuille de Pois supplémentée en poudre de feuille de Pomme de terre constitue une ration à peu près équivalente à celle de la feuille fraîche de Pomme de terre pareillement supplémentée, compte tenu d'une consommation réduite; et d'autre part que l'addition de lécithines à la poudre de Pomme de terre sur feuille de Pois triple ou quadruple la fécondité pour une consommation équivalente.

DISCUSSION ET CONCLUSIONS

Les facteurs biochimiques de la fécondité chez les Phytophages sont peu connus. Les travaux les plus récents et les plus détaillés se rapportent aux Diptères hématophages et spécialement à *Aedes aegypti* L. (LEA, DIMOND, DELONG *et al.* 1955, 1956). Ces auteurs ont déterminé les besoins quantitatifs et qualitatifs d'azote pour assurer la meilleure oviposition: ainsi 8 acides aminés sont indispensables à celle-ci et la ponte est nulle quand un seul de ces 8 acides est absent: arginine, isoleucine, leucine, lysine, phénylalanine, thréonine, tryptophane et valine. L'absence de l'histidine ou de la méthionine limite le taux de croissance ovarienne mais quelques rares oeufs sont pondus; il en est de même, à un degré moindre, pour la cystine et l'acide glutamique.

Le Diptère phytophage *Ceratitis capitata* Wied exigerait aussi un régime protidique pour assurer une ponte normale (HANNA 1938, 1947). On a pu croire qu'il en était ainsi chez la plupart des Diptères; mais des travaux récents en limitent beaucoup l'importance: d'après RASSO et FRAENKEL (1954) le développement des ovaires de *Phormia regina* Meigen est plus satisfaisant si l'on ajoute à des protéines diverses, outre les sucres, les vitamines du complexe „B" et surtout des sels minéraux notamment du phosphate de potassium; pour ASCHER et LEVINSON (1956) l'enrichissement du régime larvaire en protéines chez *Musca vicina* Macq. est insuffisant pour assurer l'ovogénèse chez l'imago.

Chez l'Orthoptère *Melanoplus mexicanus mexicanus* Sauss, SMITH et NORTHCOTT (1951) obtiennent un accroissement de fécondité en enrichissant en azote le milieu nutritif utilisé pour la croissance du Blé offert aux Insectes. Si l'on ajoute au sucre une ration de pollen et de gelée royale qui fournit de l'azote mais aussi des substances indéterminées, l'augmentation de la ponte des Reines d'*Apis mellifica* L. atteint le double de celle des témoins (CHAUVIN, 1956).

Chez le Doryphore les suppléments protidiques que nous avons apportés à la ration sous forme de caséine semblent indiquer que la fonction reproductrice exige une quantité minimum d'azote au-dessus de laquelle tout supplément est peu efficace.

Les besoins qualitatifs d'azote susceptibles de satisfaire à une ovogénèse normale ne peuvent pas être définis chez les Phytophages avant la mise au point d'un milieu artificiel convenable pour l'Insecte. Dans ce domaine, il convient de souligner la réussite récente de E. S. VANDERZANT (1957) qui a obtenu chez *Pectinophora gossypiella* Saund., pour la première fois, la croissance normale des larves et la reproduction des imagos qui en sont issus sur milieu d'agar chimiquement défini.

Les sucres offerts en suppléments au Doryphore sont remarquablement assimilés par l'Insecte et ils sont en partie stockés sous forme de glycogène dans les oeufs, bien que nous constations souvent une diminution de l'indice de fécondité, ainsi qu'en témoignent les résultats d'analyse suivants (Table VII).

TABLE VII

Aliment	*f. jeunes Bintje*	*f. jeunes + glucose*
quantité d'oeufs pour 20 ♀♀	497 oeufs	307 oeufs
poids total frais des oeufs	304,5 mg	170 mg
poids frais de 1 oeuf	0,61 mg	0,55 mg
glycogène en p. 1000 de p. frais	12,3	17,6
glucose	3,5	4,0

Nous pensons que le Doryphore doit être exigent en glucides pendant la période de maturation en vue du stockage de réserves glycogéniques mais aussi de réserves triglycéridiques ou de lipides énergétiques dans le tissu adipeux du Doryphore avant la diapause (BUSNEL 1939, GRISON et LE BERRE 1953).

Une partie des sucres doit être transformée en graisses comme l'ont bien mis en évidence FULTON et CHAMBERLIN (1934) chez *Eutettix tenellus* Baker nourri sur un régime exclusivement constitué d'une solution de 1,5% de glucose et de 1,5% de fructose; KOZHANTSHIKOV (1938) a cherché chez plusieurs Lépidoptères, comment les glucides alimentaires étaient transformés en graisses pendant la maturation pour être utilisés dans la formation du vitellus des oeufs.

Pendant la période de reproduction, le Doryphore trouve probablement une ration glucidique suffisante dans son aliment normal, ce qu'expliqueraient les résultats défavorables obtenus avec une ration alimentaire trop enrichie („surchargée") en glucose. Selon nous, on peut admettre que ce sont d'abord les réserves du tissu adipeux qui interviennent dans la fonction ovogénétique: toutes les dissections opérées au cours de la période reproductrice montrent la diminution progressive de ce tissu, pauvre en lipides phosphatidiques.

L'étude du rôle des glucides devrait donc être faite parallèlement à celle du rôle des lipides, sans négliger d'autres constituants essentiels[1]) (SCOGGIN et TAUBER, 1950). J. S. BARKER (1952) a fait varier la composition chimique de *Pisum sativum* L. en modifiant les conditions de culture et, quelles que soient les variations de teneur en azote total et en glucides, il a constaté que les lipides jouaient un rôle actif dans la reproduction de *Macrosiphum pisi* Kalt.

Pour notre part, nous avons établi l'efficacité des lipides phosphatidiques sur la fécondité de *Leptinotarsa decemlineata*; et nous avons contrôlé leur assimilation par l'organisme de l'Insecte en effectuant des „bilans choliniques" rapportés ailleurs. Rappelons cependant les données fondamentales obtenues avec des lots importants de femelles nourries avec aliment normal (f. jeunes „Bintje") et avec le même aliment supplémenté avec une émulsion de lécithines à 5 p. 100 (Table VIII).

TABLE VIII

	Lot avec f. jeunes	*Lot avec suppl. lécith.*
ponte totale pendant 10 jours . .	769 oeufs	903 oeufs
femelles fécondes	19 sur 39	21 sur 31
indice de fécondité	2	3
choline totale en mg :		
— pour 1 gr d'oeuf	1,51 mg	1,40 mg
— pour 100 oeufs	0,945 —	0,860 —
— pour la ponte de 10 jours . .	0,727 —	0,777 —
— par femelle	0,249 —	0,290 —
— par mâle	0,166 —	0,176 —

A l'inverse de ce qui se passe pour les sucres, la teneur en choline de l'unité d'oeuf de Doryphores nourris avec un aliment supplémenté est plus faible, mais l'indice de fécondité est plus élevé et sur la ponte totale la quantité de choline est également plus grande.

L'examen des ovarioles pratiqué tous les 5 jours sur des femelles recevant des

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[1]) Ainsi la thiamine, qui favorise la conversion en graisses des sucres alimentaires en excès, est rencontrée en plus grande quantité dans les feuilles jeunes des végétaux que dans les tissus âgés (HURIN, 1944).

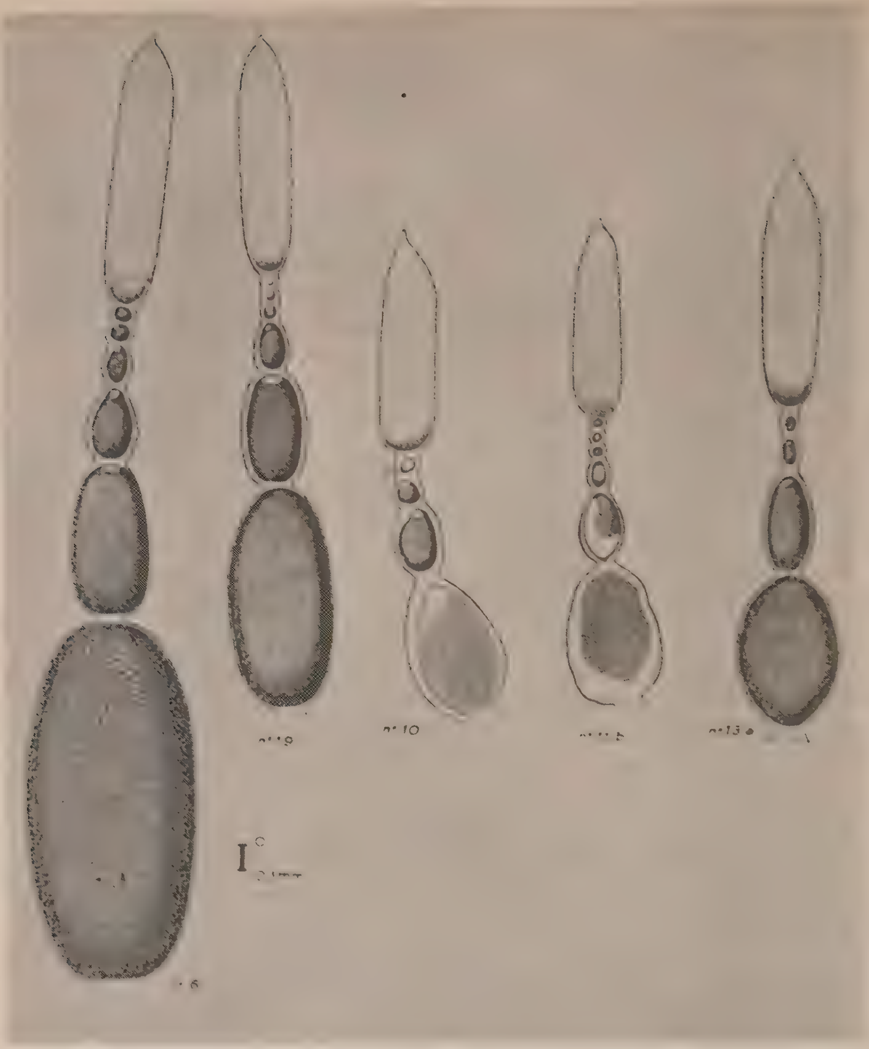

Fig. 6. Schémas des ovarioles de *L. decemlineata* au cours d'une alimentation définie.
— n° 6 après 10 jours sur F. jeune,
— n° 19 — 20 — sur F. jeune + glucose,
— n° 10 — 10 — sur F. âgée,
— n° 11 — 20 — — — —
— n° 13 — 20 — — — — puis 5 jours sur F. âgée + lécithine.

nourritures différentes apporte une nouvelle confirmation du rôle respectif du glucose et des lécithines (fig. 6)[1].

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[1] Le rôle des stérols aurait mérité d'être pris aussi en considération (de rares essais de supplémentation avec le cholestérol ont été sans résultat). Chauvin (1949) a obtenu un meilleur taux de reproduction de *Blattella germanica* L. avec un régime enrichi en stérols à faible dose. Leur rôle a été surtout étudié dans le développement larvaire et la nymphose: Hobson 1935, Fraenkel et Blewett 1943, Golberg et Meillon 1948, Bergmann et Levinson 1954, Rasmussen 1956, etc.

D'autre part l'examen des activités diastasiques a révélé une action lécithinasique très prononcée.

Nous ne savons pas quelle est la fraction assimilable des lipides phosphatidiques. Mais nous accordons une importance particulière à l'opinion émise par PEPPER et HASTINGS (1943) puis par FRAENKEL et BLEWETT (1947) sur le caractère diététique fondamental de l'acide linoléique et sur la nécessité de la présence de cet acide gras dans la plante-hôte pour assurer une fécondité normale chez *Loxostege sticticalis* L. Cet acide est, avec l'acide linolénique et l'acide arachidonique, l'un des précurseurs alimentaires indispensables des lécithines et des phospholipides car il est presque exclusivement trouvé chez le végétal et ne peut être synthétisé par l'organisme animal. VANDERZANT et REISER (1956, 1957) l'incorporent dans le millieu synthétique avec lequel ils obtiennent le développement larvaire puis la ponte des imagos de *Pectinophora gossypiella.*

En conclusion, si les variations du rapport C/N ne présentent aucune corrélation avec les fluctuations de la fécondité de *L. decemlineata,* la teneur de l'aliment normal en azote total et en glucides n'est pas indifférente à ces fluctuations. En particulier l'apport glucidique ne doit pas excéder une certaine justification physiologique du seul point de vue de la fonction reproductrice, sinon une partie de la ration glucidique est dirigée vers les tissus musculaire et adipeux et est susceptible d'accroître le potentiel d'activité motrice de l'Insecte.

Par voie de conséquence indirecte, le rythme des processus ovogénétiques est occasionnellement ralenti puisque le siège d'intense métabolisation des matières est ailleurs: on assisterait en quelque sorte à une „déviation du métabolisme" sans que le rôle des glucides soit pour autant devenu négligeable dans l'accomplissement de la fonction reproductrice chez le Doryphore.

Tandis que, normalement, l'enrichissement et les exigences de l'organisme de *L. decemlineata* en lipides phosphatidiques s'expliqueraient par le rôle de ceux-ci dans le transfert et l'utilisation des glycérides énergétiques, assuré peut-être sous contrôle endocrinien, sans que soit exclue la mobilisation d'une partie des lécithines par la fonction reproductrice et spécialement par la vitellogénèse.

SUMMARY

The fertility of oligophagous plant-eating insects varies, depending both on the species of plant eaten, and on the variety of that plant. We have investigated the effects of certain organic substances in the leaves of the potato *Solanum tuberosum* L. on fertility in the Colorado potato beetle *Leptinotarsa decemlineata* Say. By considering the ratio between the "maintenance ration" for the adult beetle, and the amount eaten during egg production, it is possible to determine the efficiency of egg production.

Causes of variation in the chemical constitution of leaves are reviewed. These include cultural conditions, time of day, stage in development, age of the leaf. Changes in the C/N ratio are less important than changes in the amount of cholin-containing lipids in their effect on the fertility of the Colorado potato beetle.

The effect of nitrogen and carbohydrates was studied mainly by adding different amounts of organic substances to potato leaves. Thus casein increased the rate of egg laying when the leaves were poor in nitrogen. The effect of adding glucose is more difficult to interpret, because of its importance in muscular activity. When glucose is provided in large quantities, some is stored in the ovaries as glycogen and this diminishes during egg production.

The importance of phospho lipides can be shown more exactly; some part of the lecithins are mobilised during reproduction and particularly when yolk is being produced.

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DISCUSSION.

J. DE WILDE : Avez-vous observé une augmentation du pourcentage d'individus en diapause en leur donnant des feuilles sénescentes comme aliment?

P. GRISON : Oui, mais pas systématiquement, car dans nos conditions d'élévage sans photopériode suffisante le pourcentage d'individus en diapause était toujours élevé.

C. ELLENBY : Have you considered the possible influence of photoperiod on your results ? This will vary with the temperature and potato variety. I have found with the potato root eelworm that the number of females is reduced on plants grown under short day conditions, and others have found fewer eggs laid by the root knot eelworm on plants grown on short day conditions. It has also been shown by VAN DEN BRANDE and his colleague that this effect varies with the wave-length of light.

P. GRISON : Les cultures ombrées sont faites sous ombrage permanent et nous obtenons du feuillage étiolé. Je remercie Dr. ELLENBY de ses suggestions et je suis persuadé de l'intérêt d'utiliser le photopériodisme et différentes lumières pour modifier le chimisme végétal. Je n'ai pas considéré les différences de réponse des „variétés" à de telles conditions et cette suggestion est également très intéressante.

A. J. THORSTEINSON : Dr. GRISON has demonstrated that lecithin increases not only fecundity but also consumption of food. Does he agree that the latter is an indication that lecithin improves the palatability of the food ?

P. GRISON : Les tests de fécondité ne sont pas une démonstration suffisante mais je pense que ce facteur du comportement peut être pris en considération dans le schéma du Prof. DE WILDE et qu'il devrait être étudié selon les méthodes exposées par DE WILDE et Dr. THORSTEINSON.

K. SCHREIBER : I should be grateful if you could tell me, how you have tested the effect of lecithin on the Colorado Beetle.

P. GRISON : J'utilise des lécithines d'oeuf du commerce qui sont émulsionnées dans l'eau distillée après avoir ajouté une goutte d'alcool 70°. L'émulsion est répandue sur la feuille par pulvérisation.

Ph. SEDEE : Did you study possible alterations in the mutual ratio of the fatty acids during metamorphosis and developing of the eggs?

P. GRISON : Je n'ai pas étudié cette question.

END OF SYMPOSIUM PAPERS

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THE QUANTITATIVE STUDY OF SOIL MEIOFAUNA

I. THE EFFECT OF SAMPLE TREATMENT ON EXTRACTION EFFICIENCY WITH A MODIFIED FUNNEL EXTRACTOR

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Construction details of the split-funnel extractor, a modified funnel method for extracting soil fauna are given, together with results of an experiment to test the apparatus, and the effect of sample treatment on extraction of Acarina from heathland samples.

Since 1905 when BERLESE first published his funnel method for the extraction of fauna from various substrates including litter and soil, there have been many modifications and improvements devised by workers concerned with quantitative studies of soil organisms. As a result of these refinements the numbers of animals recorded have greatly increased. The split-funnel extractor (MURPHY, 1950; 1956) was constructed in 1948 for use in a quantitative survey of meiofauna[1]) of heathland in north-east Yorkshire. Very large numbers of Acarina were obtained with this method, and the maximum density in this survey — 835,000 per m^2 in cultivated heathland with young *Picea sitchensis* Trautv. and Meyer in canopy — is believed to be the highest population recorded in Britain.

The same heathland was used for the investigations described here. The profile is a podzol with shallow, litter (2 cm) and raw humus (3—4 cm) horizons, and a very distinct, thin iron pan occurring some 17 cm below the surface. The dominant vegetation is *Calluna vulgaris* Salisb. A description of the habitat and the Acarina occurring there will be found in MURPHY (1953; 1954).

In addition to tests of the efficiency of the apparatus, details are given of the effect of sample treatment on the recovery of the fauna. Only the Acarina or mites are considered in this analysis. There has been no attempt to compare the split-funnel with flotation extraction as the presence of a discrete organic layer on the heathland surface, and indeed in many forest sites, precludes the use of existing flotation methods in these habitats.

THE APPARATUS

The split-funnel extractor utilizes the basic principles of BERLESE's (1905) original funnel, the use of heat to gradually desiccate the sample and thus drive out the fauna. It is based on the TULLGREN (1918) modification, and is in part adapted from a design used by FORD (1937). The apparatus consists of two parts (Fig. 1): an upper cylinder 30.5 cm *h* (12 in.) carrying the heat source,

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[1]) The meio- or mesofauna have a size range of about 100 μ—1 cm and include most Arachnida, some Myriapoda and the smaller insects.

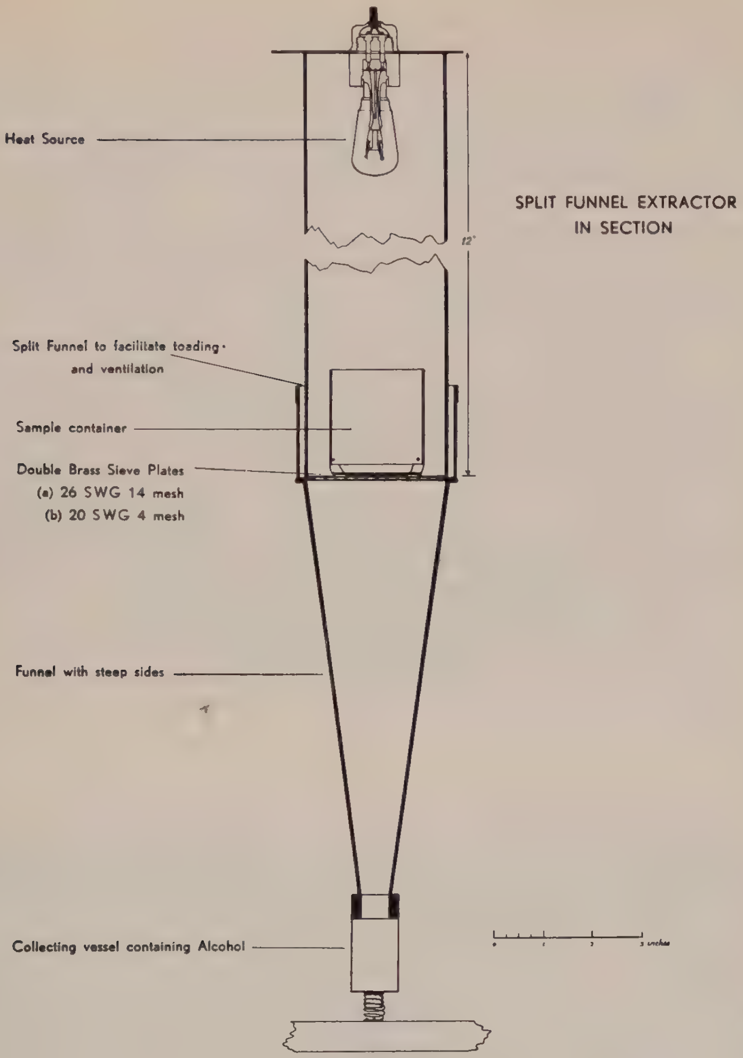


Fig. 1. Split-funnel extractor unit in section. Scale: 1 mm = 0.4 cm

and a lower funnel-shaped unit (8 cm *d* × 24 cm *h*; 3⅛ × 9½ in.) containing a wire-mesh sieve plate to support the sample container. At the funnel base (1.6 cm *d*; ⅝ in.) there is a collecting tube held in position with a rubber bung. The tube rests on a small metal spring to prevent its becoming detached from the funnel. The two units, preferably constructed in a non rusting metal such as aluminium alloy, are mounted in batteries of eighteen in a wooden and metal framework (Fig. 2) of rigid construction in order to avoid vibration. The cylinder units are supported by a metal plate counterpoised with two weights so that they can be raised and lowered, and thus allow access to the funnels for loading and cleaning. The name split-funnel is derived from this constructional feature *viz.*, the ability to separate the funnel into two component parts — the cylinder and cone-shaped tube — as illustrated in Fig. 2. When extraction is taking place the cylinders are lowered and rest within the upper parts of the funnel units.

The sample container (Fig. 3) is a metal tube 5.1 cm (2 in.) *d* and 5.1 cm *h*. At its base are 3 brackets supporting a sieve plate (26 SWG, 14 meshes per linear inch; 5.5 per cm), which rests 0.5 cm ($^3/_{16}$ in.) below the lower rim of the container. The gap between sieve plate and container rim is sufficient to allow the escape of animals too large to pass through the meshes of the container sieve plate. The container is supported on a larger sieve plate (20 SWG, 4 mesh; 1.6 per cm; Fig. 3) resting on a flange at the base of the straight-sided upper portion of the funnel unit (Fig. 1). The purpose of the double sieve-plate arrangement is to prevent debris falling into the collecting tube. The heat source is a 15 W electric-light bulb, a pigmy pattern with a small bayonet fitting. These are mounted in sets of 9 on batons with two sets for each funnel battery. This arrangement facilitates the replacement of burnt-out bulbs. The voltage of the circuit can be adjusted to provide approximately the required temperature. A thermostat is inserted in one funnel (Fig. 2) so that temperature fluctuations are reduced to ± 2° C.

The efficiency of the apparatus is due in part to the method of air circulation. In many funnel designs the air enters the lower orifice of the funnel, passes through the sample and finally escapes at the top. As the base of the split-funnel is closed, the air current must enter at the point where funnel and cylinder meet; it circulates freely around the sample container (there is a free space of 1.3 cm (½ in.) between latter and funnel wall) before passing up the cylinder, and out through ventilation holes surrounding the bulb socket. This arrangement whilst ensuring a constant change of air, creates a comparatively dead space in the funnel below the sample, and thus prevents desiccation of its undersurface in the early stages of extraction. During colder periods of the year, it is necessary to have a reasonable room temperature if the air temperature at sample level is to reach 30—35° C. It is an advantage to increase the temperature in the later stages of extraction in order to drive out more resistant organisms, as suggested by MACFADYEN (*in litt.*). A probable improvement would be to reduce the distance between heat source and sample whilst retaining the 30.5 cm (12 in.) cylinder. The apparatus should be installed in a quiet room, otherwise some sample material may fall into the collection tubes. In summer especially, insects may be attracted by the funnel lights, and in this case the battery should be covered with muslin or similar material.

To the collection tubes (2.5 cm *d* × 5.1 cm *h*; 1 × 2 in.) are added about 5 ml of alcohol solution based on a formula recommended by EATON & CHANDLER (1942), but modified by omitting glacial acetic acid. This medium is quite suitable for most mites, although for small, delicate species and also Collembola it is preferable to use GISIN's (1947) fixative. If the latter is used the specimens should be removed or the solution diluted after 2—3 days, otherwise the formaldehyde will have a deleterious effect (GISIN, 1947). The formulae are as follows:

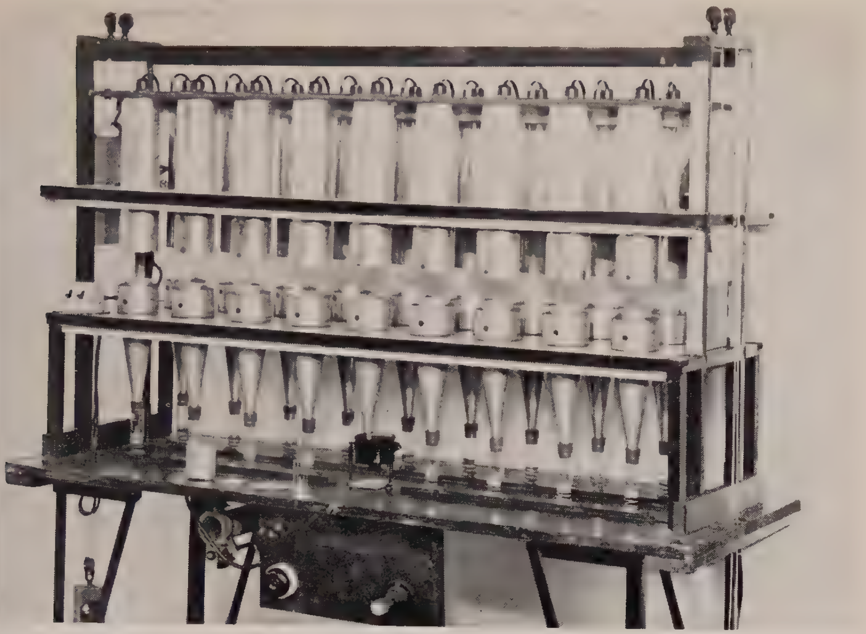


Fig. 2. Split-funnel battery in open position. Note thermostat in fifth funnel from left. *c.* $^1/_{225}$ actual size.

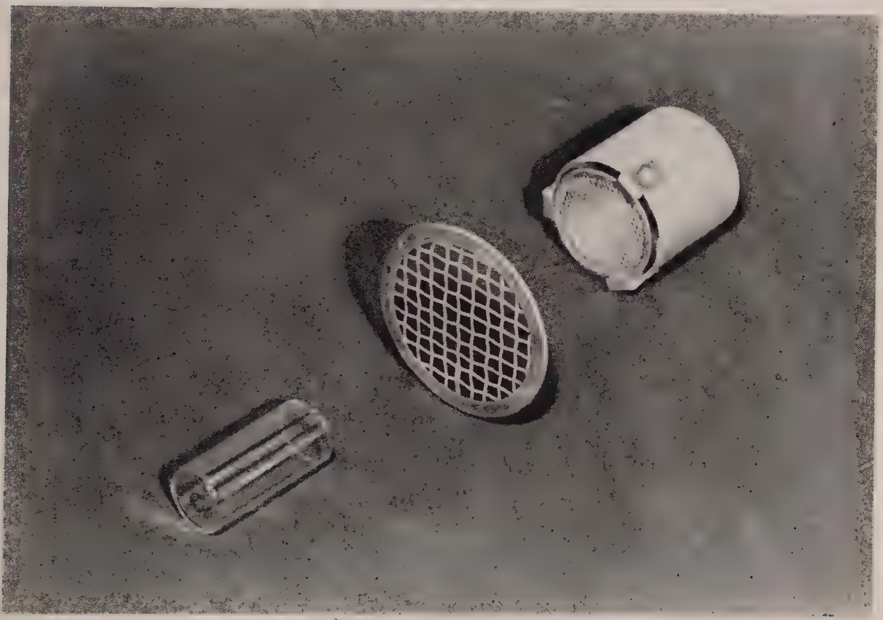


Fig. 3. Sample container, large sieve plate (4 mesh) and collecting tube. *c.* $^1/_9$ actual size

Collecting Solution (Eaton and Chandler)	
Absolute alcohol	5 parts
Distilled water	3 parts
Glycerol	1 part

Gisin's Fixative	
Alcohol 95%	750 ml
Ether	250 ml
Acetic acid	30 ml
Formaldehyde 40%	3 ml

GISIN's mixture has the serious disadvantage that it evaporates rapidly, and requires frequent replenishment. There is the additional danger that in a partially closed system such as the split-funnel the evaporating liquid (especially ether) may have a deleterious effect on the living fauna.

The principal advantages of the split-funnel extractor are as follows: the temperature is easily controlled, and desiccation of the sample is so arranged that it takes place from the top downwards, and reduces to a minimum drying of the lower surface until the later stages of extraction. The apparatus is designed for small samples (20—100 cm³), which can be easily and rapidly placed in position; there is a special sample container and double sieveplate arrangement to prevent sample debris falling into collecting tube; steepsided funnels reduce the numbers of organisms lodging on the sides; and a removable sieveplate allows ready access to funnel unit for cleaning.

EFFECT OF SAMPLE TREATMENT ON EXTRACTION EFFICIENCY

There are two related aspects to the efficient recovery of organisms: the extraction apparatus, and the method of sample preparation. There is the additional qualification that no one method extracts all the fauna, or for that matter all the meiofauna (MACFADYEN, 1953). TRÄGARDH (1933) was the first to analyse the funnel process, and he considered that desiccation (apart from a small negative phototactic response) was the principal factor involved. HAARLØV (1947) confirmed the majority of TRÄGARDH's findings; and also suggested that in addition to desiccation, temperature provided a direct stimulus at a certain stage in extraction. The importance of a humidity gradient was implied in TRÄGARDH's experiments, and MACFADYEN's (1953) study, utilizing a highgradient funnel, offers convincing evidence that extraction efficiency is directly related to steep, temperature and humidity gradients within the sample.

Even a superficial analysis of sample treatment and its effect on the fauna suggests that sample disturbance, depth and orientation are important in deciding the efficiency of the process. JACOT (1936) considered that best results were obtained when the material was extracted in thin layers in an undisturbed condition, with the samples placed surface downwards in the funnels. Some investigators have assumed that these requirements are essential; MACFADYEN for example, made the initial assumption that undisturbed, inverted samples were necessary for good extraction results. Thus apart from one small experiment by VAN DER DRIFT (1951) on the effect of disturbance, and MACFADYEN's inconclusive test of the effect of subdivision, there has been, as far as the author is aware, no published account of the influence of these factors.

It is usually considered that the interval between sample collection and extraction should be as short as possible. With delayed extraction there may be changes due to breeding, moulting and mortality. Breeding may have an important effect on the numbers of Trombidiformes, but is unlikely to influence the Oribatoidea as very few larvae and relatively few nymphs are recovered. Besides with Oribatid mites, the time taken to reach the active, protonymphal stage from a newly-hatched egg is about 13 days with *Scheloribates laevigatus* Koch (temperature 25° C; CLEAT, 1952), which rules out this factor unless an unusually long period elapses between collection and extraction. Juvenile oribatid mites have an immobile developmental phase before each nymphal stage (JONES, 1950) lasting about three days in each case (*S. laevigatus*; CLEAT, 1952) and if this takes place during extraction it will result in under- or over-estimation of active nymphs. Where there are short delays, mortality appears to be the most likely factor affecting the numbers of Oribatoidea recovered.

EXPERIMENTAL PROCEDURE

In the experiment reported here the following treatments were compared:

1. Litter and raw humus extracted separately
2. Sample subdivision
3. Sample disturbance
4. Sample orientation
5. Extraction at room temperature
6. Delayed extraction

Five blocks 15 × 15 cm (6 × 6 in.) and 5—7.5 cm (2—3 in.) deep, consisting of litter, raw humus and a little mineral soil were obtained from a Yorkshire heathland in November, 1949. They were taken from a 1.8 m (6 ft.) transect in a part of the heathland having a very uniform *Calluna* cover. The blocks were transported to Oxford, and after 4 days, 2 of them at an average room temperature, undisturbed cores were cut (see MURPHY (1958) for details of method). Table IV summarizes the sample treatments. The 'standard' treatment consisted in removing the litter first, cutting an undisturbed core and dividing it into a number of subsamples, each 1.3 cm (½ in.) deep. The sample volumes excluding litter were 20—25 cm^3 with a surface area of 17.8 cm^2. The litter was extracted separately at a temperature of 20—25° C, the raw humus and soil at 30—35° C. Only deviations from the standard treatment are listed in the appropriate row of the table. The disturbed sample was prepared by carefully cutting up an undisturbed core, 5 cm (2 in.) deep including litter, with a scalpel, and placing in 2 containers with litter at bottom of first (depth of samples in containers respectively 5 and 2.5 cm). The samples were loaded on the fifth day, and extraction completed 6 days (135 hours) later. Room temperature during extraction was *c.* 16—20° C. The subsamples for delayed extraction were prepared in the same way and at the same time, wrapped in cellophane and stored in a tight-fitting tin in a refrigerator (*c.* 4° C). Extraction commenced on the twelfth day after sampling and was completed 7 days (169 hours) later. In all cases the samples were weighed and collection tubes changed at intervals during extraction, in order to correlate movement out of the samples with moisture content; the latter is expressed as a percentage of the fresh sample volume (Fig. 5).

PRETREATMENT TO STANDARDIZE SAMPLES

To overcome the difficulties created by the irregular distribution of the fauna from sample to sample, two courses are open to the investigator. A large number of samples may be extracted and the results statistically analysed; or if a small number are dealt with, a means must be devised to reduce the inherent variation between samples. The latter course was adopted in the experiment described here. The method used was to maintain the blocks for about 2 days at an average room temperature, before preparing the samples. This treatment would cause a certain amount of drying to take place, and it was believed that the fauna would tend to concentrate in the centre of the block, and thus iron out local aggregations, which might otherwise mask effects due to sample treatment.

TABLE I

TOTAL NUMBERS ACARINA PER SAMPLE

Sample number	*Nanorchestes arboriger*	Other Trombidiformes	Oribatoidea	Undetermined and other Acarina *	Total excluding *N. arboriger*	Grand total
1	492	74	241	114	429	921
2	371	75	141	21	237	608
3	95	43	70	75	188	283
4	43	37	48	39	124	167
5	571	72	123	38	233	804
6	419	74	80	24	178	597
7	25	65	100	16	181	206

* Includes Mesostigmata, Acaridoidea and some unidentified specimens (principally Trombidiformes).

In order to find if there was in fact a movement from edge towards centre, two samples were taken from the centre of block 1, and two from the edge of block 2 (Tables I and IV). Comparing samples 1 and 3, it is obvious that there is a very large reduction in numbers in the latter, despite the fact that the only treatment difference is orientation (sample 1 inverted), and as will be shown later, it is unlikely that this factor could have such a marked effect with shallow subsamples. The reduction is especially noticeable with *Nanorchestes arboriger* Berl. (Endeostigmata; Table I), a small, active, saltatorial mite which appears to be rather sensitive to extraction conditions. A further check on the method is to find if the variation between samples has been reduced. The only samples, directly comparable, are the litters of Nos. 1 and 5, and there would seem to be little agreement, the totals being 380 and 249. If however these are expressed as numbers per gramme extraction-dried litter (Table II), the figures 199 and 162 show better agreement. The numbers of individual groups are very similar except in the case of *Carabodes minusculus* Berl. (Oribatoidea; Table II), and it is clear that there is a very marked aggregation of this species in sample 1 and to a lesser extent, sample 2 (Fig. 4). Table IV gives the percentages of Acarina excluding *N. arboriger* and *C. minusculus* in samples 1, 2 and 5, and these agree

TABLE II
NUMBERS ACARINA PER GRAMME EXTRACTION-DRIED LITTER

Sample number	Litter weight/g	*Nanorchestes arboriger*	Other Trombidiformes	*Carabodes minusculus*	Other Oribatoidea	Undet. and other Acarina *	Total excluding *N. arboriger* and *C. minusculus*	Grand total
1	1.91	91	13	41	35	19	67	199
2	2.09	95	15	19	36	6	57	171
3	1.63	45	15	11	21	15	51	107
4	1.44	17	19	7	17	15	51	75
5	1.54	91	18	10	29	14	61	162

* See footnote Table I

closely despite a treatment difference. The close correspondence in numbers of oribatid nymphs in all five litter samples is quite remarkable (Fig. 4), the figures being respectively, 15, 16, 14, 15 and 14. In view of the above evidence and excluding *C. minusculus,* the most abundant oribatid mite, the distribution of which is such that some of the blocks do not contain a centre of high population, it seems reasonable to assume that the inherent variation between samples has been reduced.

TABLE III
NUMBERS ACARINA IN RAW HUMUS AND MINERAL SOIL

Sample number	*Nanorchestes arboriger*	Other Trombidiformes	Oribatoidea	Undetermined and other Acarina *	Total excluding *N. arboriger*	Grand total
1	318	50	95	78	223	541
2	173	43	26	9	78	251
3	21	19	18	50	87	108
4	18	9	14	18	41	59
5	431	45	63	16	124	555

* See footnote Table I

QUANTITATIVE EFFECTS OF SAMPLE TREATMENTS

Table IV outlines the principal treatment effects. Taking the combined totals for litter and raw humus first, the results can be divided into four groups: 'standard' treatment is highest (sample 1); room temperature and deep subsamples come next (samples 2 and 5), followed by sample 6 with deep subsamples and litter not separated, and 7 as last but with raw humus disturbed; and finally delayed extraction (sample 4). From this analysis one can conclude that 'standard' treatment gives best results, and that litter and raw humus extracted together has an adverse effect on the numbers recovered.

Separating the litter and raw-humus totals (Tables II and IV) reveals interesting results in the case of the litter subsamples. 'Standard', room temperature and delayed-extraction treatments show small differences, the totals being 67, 57 and 51 (edge sample). It is clear that room temperature, and rather surprisingly, delayed extraction (apart from *N. arboriger*), have not affected the numbers recovered to any significant extent. However it must be borne in mind that the standardization pretreatment would tend to filter out more sensitive species in all litters, an effect that should be most marked with the edge samples.

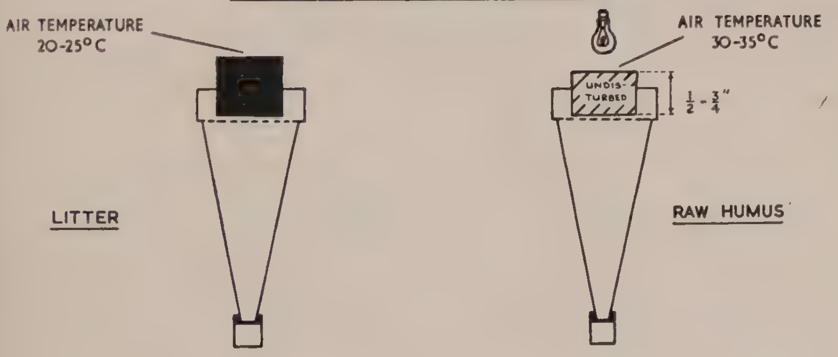
TABLE IV

SUMMARY OF SAMPLE TREATMENTS AND RESULTS

(Total numbers Acarina per sample expressed as a percentage of total population of all samples (excluding Nanorchestes arboriger))

SAMPLE — POSITION	FROM CENTRES OF BLOCKS					FROM EDGE OF BLOCK	
SAMPLE — NUMBER	1	2	5	6	7	3	4
SAMPLE — TREATMENT & DEVIATIONS FROM 'STANDARD' SAMPLE	'STANDARD' SAMPLE *	ROOM TEMPERATURE	RAW HUMUS SUBSAMPLE 1½-2" DEEP			'STANDARD' SAMPLE	DELAYED EXTRACTION
				LITTER + RAW HUMUS TOGETHER			
				*	DISTURBED		
SAMPLE MATERIAL	← — —	— — —	PER CENT	— —	— — →	PER	CENT.
LITTER AND RAWHUMUS 1% = 16 INDIVIDUALS	27·3	15·1	14·8	11·3	11·5	12·0	7·9
LITTER % PER GRAM EXTRACTION-DRIED LITTER (CARABODES MINUSCULUS EXCLUDED) 1% = 3 INDIVIDUALS	23	20	21	-	-	18	18
RAW HUMUS 1% = 6 INDIVIDUALS	40	14	22	-	-	16	7
BLOCK NUMBER	1		3	4	5	2	

'STANDARD' SAMPLE EXTRACTION



* SAMPLES INVERTED IN FUNNELS

When the figures for raw humus alone are compared (Tables III and IV) there is no doubt that the 'standard' sample is best. The other treatments: deep subsamples, room temperature and delayed extraction, have had adverse effects in that order. It is possible to obtain approximate values for the raw humus of samples 6 and 7 by calculating a mean value for litter from the data of samples 1, 2 and 5. Then the 'standard' sample will represent 37 per cent. of the total (1 per cent. = 6 individuals), and samples 6 and 7, 3 and 4 per cent. respectively. It is necessary to assume that there is no marked reaction between presence of litter and a deep subsample on the one hand, and both coupled with disturbance on the other. With the latter there is almost certainly a reaction in the case of *N. arboriger,* but this species is excluded in calculating the above percentages. However any reaction effect can hardly account for the great disparity, and one must assume therefore that disturbance in itself has had a marked effect on the raw-humus fauna. This disturbed sample however is rather an extreme case owing to the depth of material in the container (5 cm), a fact which may exaggerate to some degree the disadvantages of this method of treatment.

The effect of orientation must now be considered (Table IV). Samples 1 and 6 are the only inverted ones, that is placed base upwards in the funnels. With sample 7, apart from litter being placed at the bottom of the container, no orientation is possible. Although this factor could be responsible for the differences between samples 1 and 5, it is unlikely as deep subsamples have given consistently poor results. When inversion is superimposed on deep-subsample treatment, then inversion and/or litter presence are responsible for a further reduction in numbers (samples 5 and 6), and when litter presence is common to both samples, inversion or disturbance results in the numbers recovered being almost the same (samples 6 and 7). It is unlikely therefore that the big difference between samples 1 and 5 can be explained on this basis. The other important comparison is between samples 1 and 3, but it is hardly possible that inversion could account for such a big difference with shallow subsamples. The effect of orientation has not been proved in this experiment, but in view of the consistently poor recovery from deep subsamples, and the fact that inversion does not appear to have an adverse effect, it is probable that the distance travelled by the fauna in the desiccating medium is of vital importance, and therefore on commonsense grounds, it is recommended that the 'standard' treatment should include subsample inversion.

FORSSLUND (1947) found large numbers of organisms remaining on the funnel sides, and despite the writer's steeper funnels (15° included angle) considerable numbers were found with certain samples. With samples 1, 3 and 4, 15—20 per cent. of the population belonged to this category; with the others the numbers were < 5 per cent. They were greatest in those samples where egress was most rapid, which suggests that heat or desiccation had something to do with it. A possible explanation is that temperature had a greater direct influence with the first group (all shallow subsamples). Very few specimens were found on the sieve plates.

HAARLØV (1947) found that animals can be trapped in drops of condensed water on the sides of glass funnels, and his design embodies features to prevent this taking place. The writer has not observed condensed water in his funnels, and it is most unlikely that this would have occurred during extraction, as the funnels used here were made of tin and would have rusted badly in consequence.

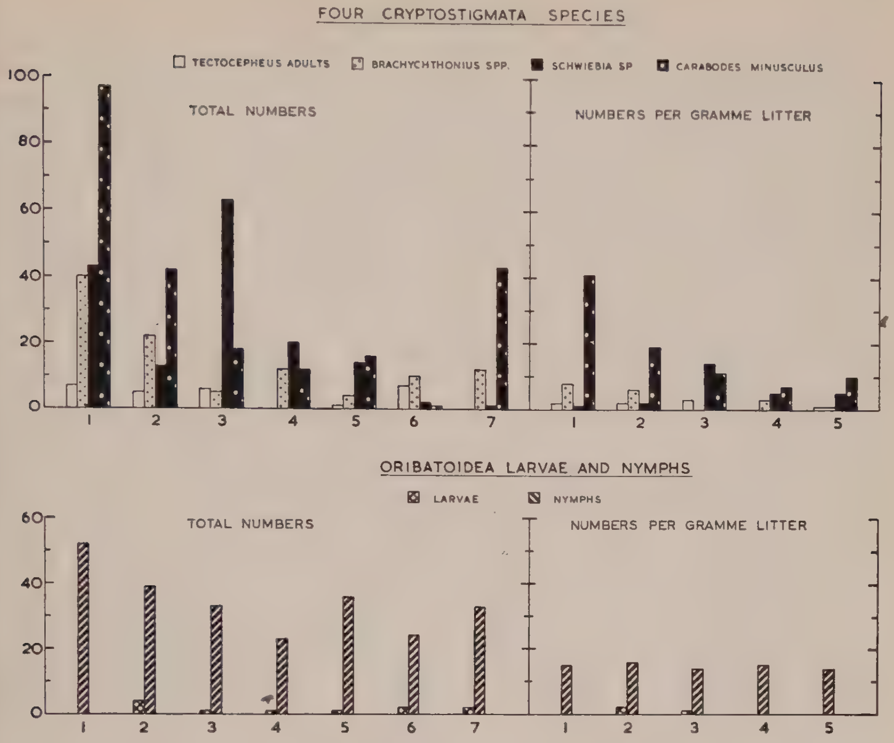


Fig. 4. Numbers of four Cryptostigmata genera, Oribatoidea larvae and nymphs. Total numbers per sample on left; numbers per g extraction-dried litter on right.

QUALITATIVE EFFECTS OF SAMPLE TREATMENTS

The small, delicate Trombidiformes and juvenile stages of the Oribatoidea are usually regarded as most sensitive to the extraction process. In the experiment described here the numbers of Trombidiformes (excluding *N. arboriger*) — principally Tydeidae and some Scutacaridae — show good agreement in all samples with the exception of delayed extraction (Tables I—IV). The population range in the five samples 1—2 and 5—7 is 65—75, but these figures will be affected to some extent by the undetermined Acarina column. Delayed extraction of the raw humus has caused a severe reduction, but rather surprisingly, the other treatments appear equally good. Many of the Trombidiformes are active mites and it is possible that this counteracts to some extent the treatment effect.

N. arboriger, also an active species, is readily influenced by disturbance, delayed extraction, and room temperature in that order. It also appears to be sensitive to either rate of desiccation or high temperature. The raw humus of samples 2, 5 and 1 representing an increasing temperature sequence yielded respectively 19, 47 and 34 per cent. of the total for all three samples (Table III). These differences are hardly due to patchy distribution as the standardization pretreatment appears to have been particularly successful with this species. The results suggest that the temperature and desiccation conditions were optimal in sample 5: this is the only instance where the 'standard' sample compares unfavourably with the rest.

The samples were taken in November, at a time when one would expect an abundance of oribatid nymphs, but the juvenile stages of some species were not represented, and it is likely that in at least certain cases absence of nymphal forms is due to the migratory habits of the adults seeking oviposition sites in other substrates (RIHA, 1951). The nymphs occurring most abundantly belonged to the species *Tectocepheus velatus* (Mich.). There was considerable variation in total numbers, the range being 24—52 (Fig. 5). Reference has already been made to the remarkably even distribution in *all* litter samples (Fig. 5), but these are probably the more resistant members. In raw humus the 'standard' sample is highest with 5 next. It is very interesting to find that the nymphs tend to be slower than adults in leaving the samples, and the same trend is observable in HAARLØV's data for nymphs of *Achipteria coleoptrata* (L.) (*Notaspis coleoptratus* (L.)) and *Platynothrus peltifer* Koch. He considered that oribatid nymphs and larvae of these species are not less resistant than adults to the desiccating process. VAN DER DRIFT (1951) had the same experience, and present results certainly corroborate this view point. Nymphs, as a rule, move much more slowly than adults, and thus are exposed to deleterious conditions for a relatively longer time. This would appear to be the crucial factor in determining the numbers recovered.

Very small numbers of oribatid larvae were collected, the total for all samples being 11 (Fig. 5). It is possible that rate of desiccation or a high temperature has had a deleterious effect, as the room temperature sample, with 4 larvae, represents the maximum recovered. The extracting conditions may have been too severe in the other samples, except perhaps sample 7 where 2 oribatid and 3 prostigmata larvae (total all samples = 4) were obtained. The absence of larvae in sample 1 and the evidence concerning *N. arboriger* would suggest that here temperature and desiccation rates were maximal, and that it would be unwise to make the process more rapid.

The Oribatid adults, usually regarded as the most readily extracted mites show greater fluctuations in numbers than any other group, the range (omitting samples 3 and 4) being 80—241. This is partly due to the massed occurrence of *C. minusculus* in some samples. It is probable that the intrinsic between-sample-variation is greater amongst the Oribatoidea than the other groups because of the presence of a relatively large number of species (at least 12), and this feature may complicate the issue. There is no doubt that deep subsamples with litter and disturbance, have had a deleterious effect (Table I); and with raw humus, room temperature has also given a very poor recovery (Table III). There is an indication that some treatments, especially disturbance have favoured certain members, particularly the Phthiracaridae and *Melanozetes mollicomus* (Koch.); the latter because of its unusually long setae may have difficulty in escaping from an undisturbed sample.

INFLUENCE OF DESICCATION AND TEMPERATURE ON THE EXTRACTION PROCESS

Present evidence suggests that temperature and rate of desiccation are mainly responsible for the movement of fauna out of the sample. Fig. 5 illustrates the rates of water loss in the shallow (sample 1) and deep subsamples (samples

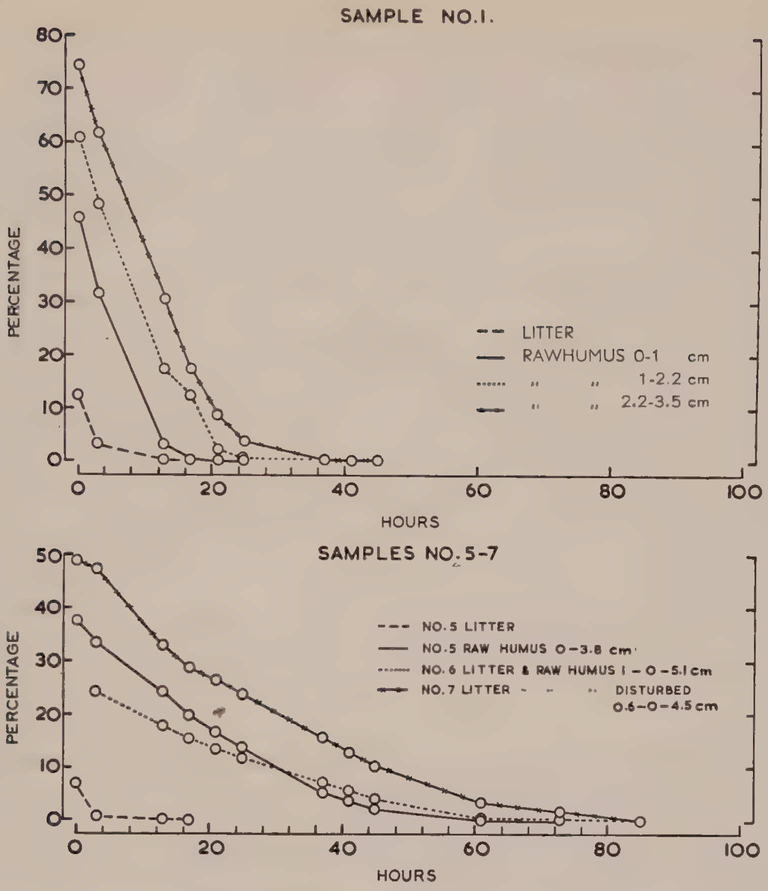


Fig. 5. Rate of drying of samples 1 (top) and 5—7. Volume water expressed as percentage of fresh sample volume.

5—7), and indicates that the initial moisture content can be very high, the maximum is over 70 per cent. of the fresh sample volume. The time taken for this to reach a 3—4 per cent. level ranges from 13 (sample 1) to *c.* 60 hours (sample 7). From the point of view of the fauna, the relative humidity of the pores and cavities will be the most important criterion, but the writer has no data for this attribute.

The times taken to recover 95 per cent. of the fauna from the raw humus of samples 1 and 5—7 were respectively 17, 40, 60 and *c.* 135 hours (Fig. 6), so that with the exception of the last, egress is closely correlated with desiccation level. In the 'standard' sample it is very rapid, almost 60 per cent. leaving the sample in the 4 hours *after* the moisture content had fallen below *c.* 3—4 per cent. The desiccation rates of the deep subsamples are very much slower (Fig. 5), and movement out of them is spread over a much longer period. This suggests that the fauna is not congregating in the base, but as the desiccation front advances

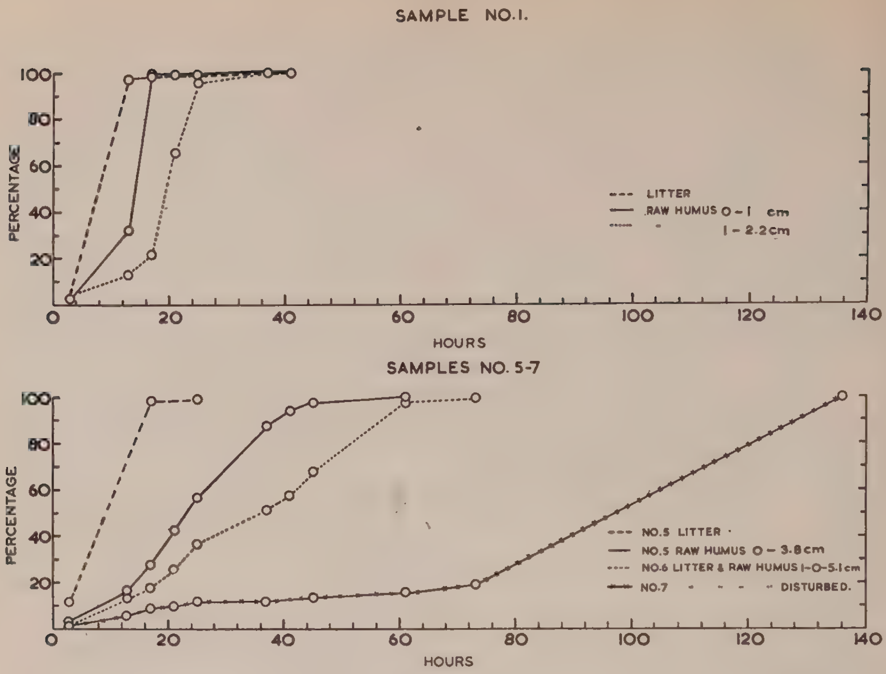


Fig. 6. Rate at which Acarina leave samples 1 and 5—7. Data expressed as a cumulative percentage.

through the material, they move horizontally to the sides rather than down through the sample, and then proceed downwards on the outer surface. A complicating factor is the very great shrinkage occurring during drying (25—40 per cent. of the fresh volume), and this may be the reason why the organisms do not appear to move down through the sample.

The presence of litter (sample 6) tends to delay egress, and may have the effect of a barrier hindering an animal's progress when it has left the raw humus. The results of the disturbed sample (No. 7) are perhaps the most interesting (Fig. 6) as here the population takes twice as long to leave although the absence of a collection-tube change between 73 and 136 hours probably exaggerates this effect. However the results leave no doubt that the fauna take very much longer to leave this sample, and the presence of litter cannot be the only cause of the delay. The absence of natural channels may be a reason although the volume of large cavities will be greater than in undisturbed samples. A more likely explanation is that isolation in individual crumbs, and the lack of temperature as a directional stimulus, owing to the mulch-like properties of the upper desiccated portion of the sample, prevent good recovery.

HAARLØV (1947) has already suggested that temperature in itself may be a factor in the process, and there is evidence from the present experiment to support this view. The rapid movement out of shallow subsamples with 'standard' treatment, and the relatively long period of desiccation tolerated by some of the fauna in sample 7 are examples of the effects of presence and absence of this factor. The room-temperature sample is a further instance of the adverse effect of absence of temperature stimulation. There is of course a limit to the amount of heat that can be applied, and it would appear that with the shallow subsamples, it was reaching a maximal value. The recovery of larvae from samples 2 and 7, and their absence in sample 1 (Fig. 4), together with the reduction of *N. arboriger* individuals and the large number of specimens recovered from the funnel sides in the latter sample, are indications that the temperature level in sample 1 was only just compatible with extraction efficiency.

CONCLUSIONS

Analysis of the results of this investigation indicates that 'standard' treatment (Table IV) gives the best recovery of Acarina. With this treatment, litter and raw humus are extracted separately, the latter in thin layers (1.3—1.9 cm; 0.50—0.75 in.) in an undisturbed condition. It should be stressed that these results were obtained with *Calluna* heathland samples and do not necessarily apply to other habitats. There is a danger, not always appreciated, of grossly underestimating populations or even species occurrence, due to establishing extraction conditions on the basis of tests of one individual habitat, and applying the results over a wide range of conditions.

There is some evidence that individual treatments have a species-differential effect with some oribatid mites. On this account it might be wise to test the effect of a number of treatments, e.g. shallow and deep subsamples, and disturbance, at varying desiccation rates when commencing a study of a particular habitat, before committing oneself to a particular sample treatment. It is possible too that a development of the concept of differential treatment — there are a number of possibilities not considered here — might make the funnel method a more versatile tool than present evidence would suggest.

MACFADYEN has made a strong case for the provision of steep temperature and humidity gradients within the sample, and the results of sample 7 particularly, indicate that these are important with *deep* samples. However the great superiority of shallow subsamples, and the virtual impossibility of achieving *steep* gradients in these when they are 1.3—1.9 cm deep, suggest that this method can be of little benefit in influencing extraction efficiency with substrates of the type used here.

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ZUSAMMENFASSUNG

Der 'geteilte-Trichter' Ausleseapparat, eine Abänderung der Tullgren-Trichtermethode zur Auslese der Bodenfauna, sowie ein Versuch zur Überprüfung der Apparatur und der Einfluss der Behandlung der Proben auf die Wirksamkeit der Auslese werden beschrieben. Bei Heidesandproben werden die besten Resultate erhalten, wenn Streu und Auflagehumus getrennt ausgelesen werden, der letztere in ungestörten, dünnen Schichten (1,3—1,9 cm). Der Unterschied zwischen Zimmertemperatur und der erwärmter Trichter (Lufttemperatur 30—35° C) hinsichtlich der Auslese der Streu ist unbedeutend; beim Auflagehumus ist die Erwärmung des Trichters jedoch wirkungsvoller.

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THE QUANTITATIVE STUDY OF SOIL MEIOFAUNA
II. SAMPLE PREPARATION AND ROUTINE METHODS FOR HANDLING THE CATCH

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Details of a method for preparation of undisturbed samples for extraction with a modified funnel are given, together with techniques for the quantitative assessment of the catch (especially Acarina and Collembola). These techniques include handling, visual examination and storage of the fauna.

In recent years increasing attention has been paid to the meio- or mesofauna of the soil, but there is very little information concerning suitable methods for use in this form of research. Those who have made a quantitative survey of soil animals well know the labour involved in the mere counting and identification of the fauna. This is due in no small part to the fact that methods have been borrowed from the entomologist, but the meiofauna which in respect of size (*c.* 100 μ—1 cm) at least, are more closely allied to the larger microorganisms, and do not readily lend themselves to these rather coarse entomological procedures. It is becoming increasingly evident that new methods must be developed if further progress is to be made, and on this account it is felt that details of some techniques evolved during the course of a study of a heathland habitat in north-east Yorkshire may prove of interest, especially to those who are venturing into this field for the first time. It is further hoped that this account of a few simple methods will encourage other workers to devote some attention to the development of suitable experimental tools for studies of this type.

The techniques outlined here concern sample preparation for extraction, and quantitative assessment of the extracted fauna. In a previous paper (MURPHY, 1958) it has been shown that method of sample preparation has an important bearing on the efficiency of the extraction process when a funnel-type extractor is used. With heathland samples best results are obtained when litter and raw humus are extracted separately, the latter in thin layers (1.3—1.9 cm; 0.5—0.75 in.) in an undisturbed condition, and the preparation treatment described here provides samples that fulfil these conditions. This method has the added advantage that there is no danger of compaction, the samples have a definite, known volume, and can be subdivided accurately according to the soil horizons. The method and apparatus for the extraction process has been described in the paper already referred to. Techniques for dealing with the extracted material are concerned with handling, visual examination and storage of the fauna.

BLOCK REMOVAL IN THE FIELD

The block method of sample removal, to provide undisturbed samples, has been devised for stony soils where the conventional type of tube sampler (COILE, 1936; MACFADYEN, 1953) will not function efficiently. The method, which consists in cutting out a square block (13 cm; 5 in. sides) of undisturbed soil from one side of a profile pit, is a modification of the technique used by pedologists to obtain soil monoliths. Although tedious it has the advantage of providing completely undisturbed samples, of considerable depth if required. There is no compaction and the samples have a definite, known volume.

A 45 × 30 cm (18 × 12 in.) pit is dug somewhat deeper than required sample depth. The vegetation on one of the pit's longer sides is cut, and a metal marking-out plate placed in position on the surface (Fig. 1). With the aid of a scalpel, a cylinder 5.1 cm (2 in.) *d* × 5.1 cm *h*, is inserted in a circular hole in the plate; it is held in position by three wire pegs, and covered with a cellophane circle secured with a rubber band. The purpose of the cylinder is to mark sample position; to prevent loss and desiccation of the litter during transport to laboratory; and to assist in final preparation of sample core. Utilizing the slots in the marking-out plate, 6 vertical slits are cut in the surface of the soil with a knife, a linoleum cutter is very suitable for this purpose. The plate is removed, and a trench 6.5 cm (2.5 in.) wide, outlined by the slits, is excavated on 3 sides of the sample block. The most useful tools for this operation are a slater's hammer, linoleum cutter, soil knife, trowel, and secateurs for cutting roots. The excavation is continued until the required depth is reached, the block is cut through horizontally at its base, lifted out, and placed in a container for transport to the laboratory.

SAMPLE PREPARATION FROM THE BLOCK

As soon as possible after removal, a sample core 5.1 cm *d* (2 in.) is cut from the block; its position is indicated by the metal cylinder inserted during block preparation in the field. First the litter is removed from the surface, care being taken not to disturb the portion within the sample cylinder. The sample litter is then placed in a carton (Fig. 3*a*). Using the cylinder as guide, a core is cut with a scalpel (Fig. 2), the surgical pattern with a detachable blade is particularly suitable for this purpose. In addition a small hacksaw is useful for trimming the humus surface, and scissors and wirecutters for cutting roots. With practice it is possible to cut sample cores with little variation in diameter. The completed core is cut up transversely into subsamples (Fig. 3 *c*), the depth of which will depend on the dimensions of the soil horizons, the maximum depth being not greater than 2 cm (0.75 in.). When the sample is very wet, and where there is a high clay content, it is necessary to take precautions to prevent the cut surface becoming smeared and pasty. Under these conditions it is better to subdivide the sample with two sharp needles rather than a scalpel. They are wrapped in plastic skin secured with rubber bands (Plate 3 *e*), and identification label inserted, which also indicates sample orientation. The subsamples are then placed in tins (Fig. 3 *d*) ready for extraction.

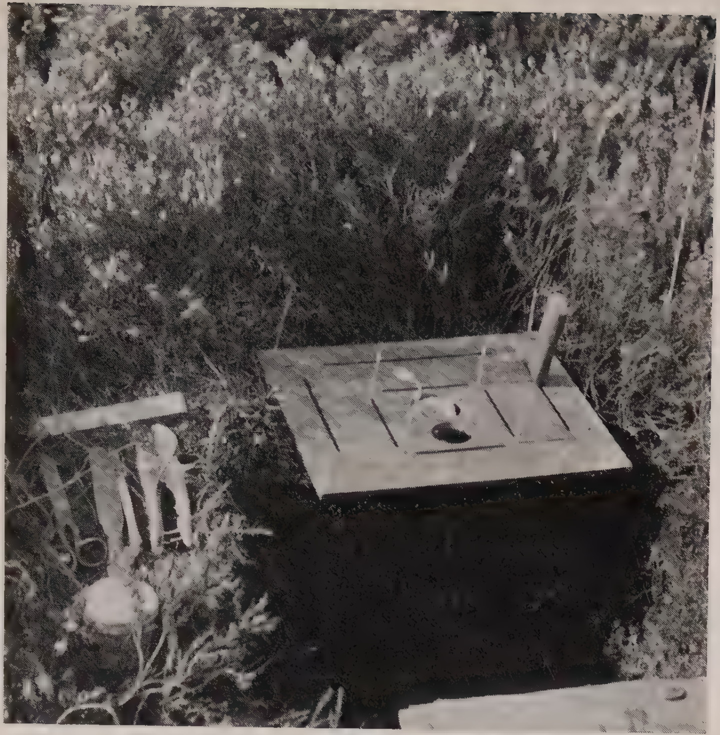

Fig. 1. Block removal in natural heathland showing marking-out plate. Sample cylinder is in position, and linoleum cutter (right) has been inserted in one of the slits to mark out block and excavation trench. The wire stakes keep plate in position. *c.* $^1/_{64}$ actual size.

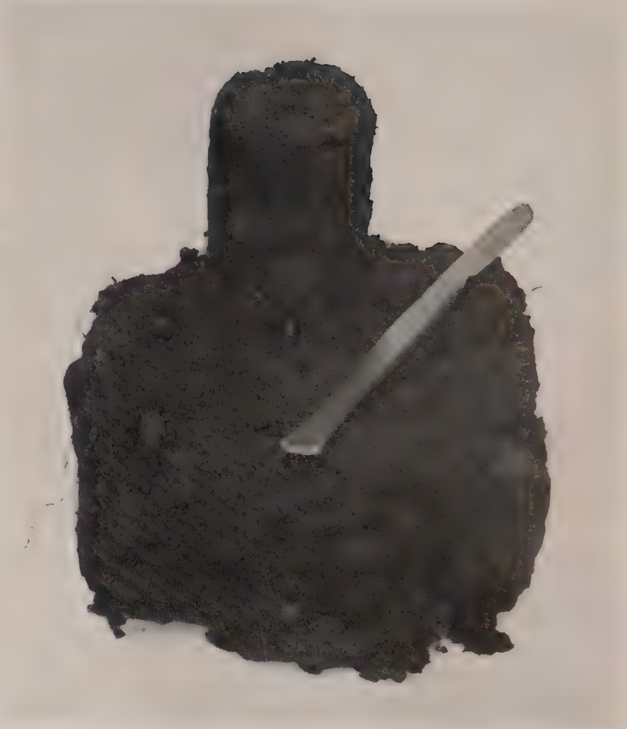

Fig. 2. Sample preparation partly completed. Block: 13 × 13 × 13—15 cm *h*; sample 5.1 cm *d*.

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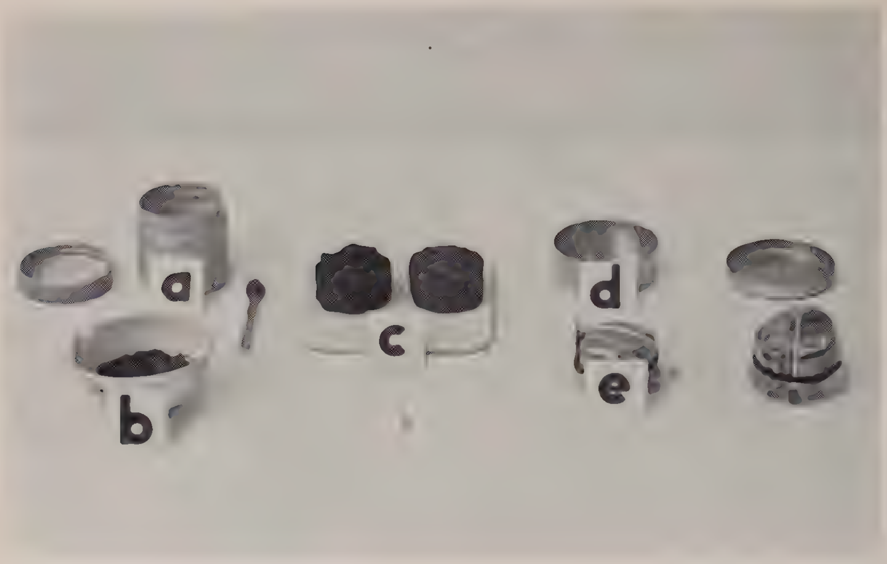

Fig. 3. Sample preparation completed. *c.* $^1/_{16}$ natural size. *a*, litter carton with lid; *b*. litter sample; *c*, raw-humus subsamples; *d*, aluminium subsample tin with lid; *e*, subsamples wrapped in plastic skin ready for placing in tins.

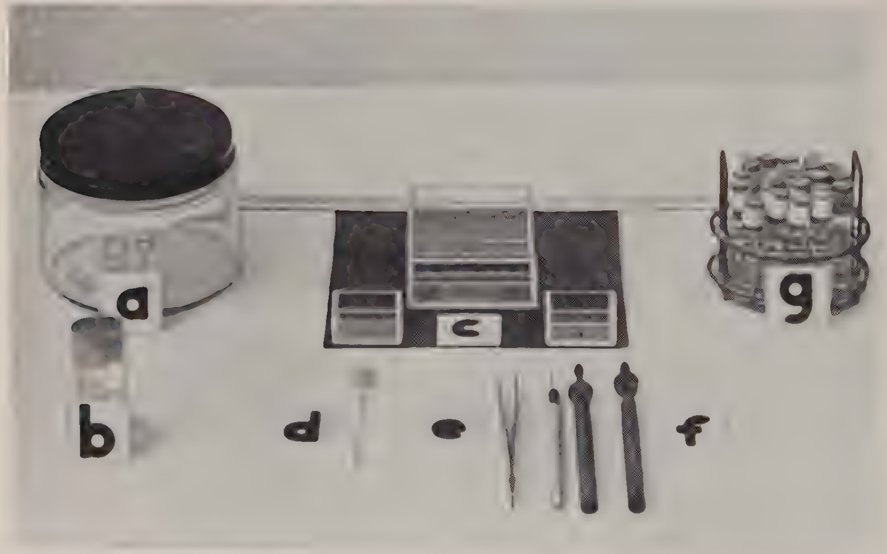

Fig. 4. Accessories for handling and storage of the catch. *c.* $^1/_{16}$ natural size. a, storage jar for wire basket (*g*); *b*, collection tube with sealed cork; *c*, counting dishes of various sizes; *d*, micro-immersion filter; *e*, fine forceps for handling specimens; *f*, needle holders and needles for the same purpose; *g*, wire storage basket holding 25 sealed specimen tubes.

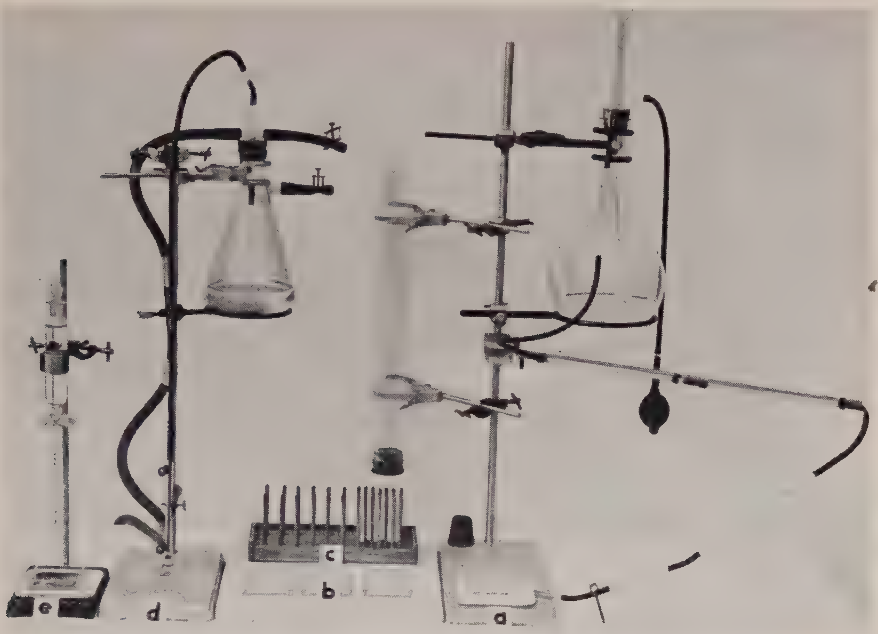

Fig. 5. Apparatus for preparation of the fauna for microscopic examination, *c.* 1/100 natural size. *a*, alcohol reservoir with automatic pipette; *b*, square counting dishes (6 × 6 × 1 cm deep); *c*, storage rack for dishes; *d*, micro-immersion filter connected to filter flask and lead to suction pump; *e*, separating funnel with glycerol.

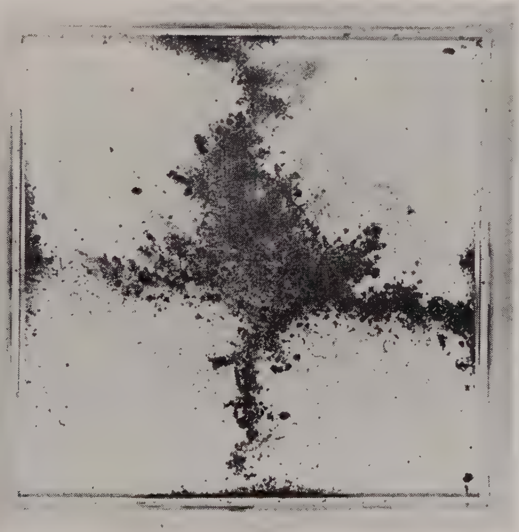

Fig. 6. Dish prepared for microscopic examination, illustrating the way in which the glycerol sweeps contents towards centre. The black debris has been added to illustrate method.

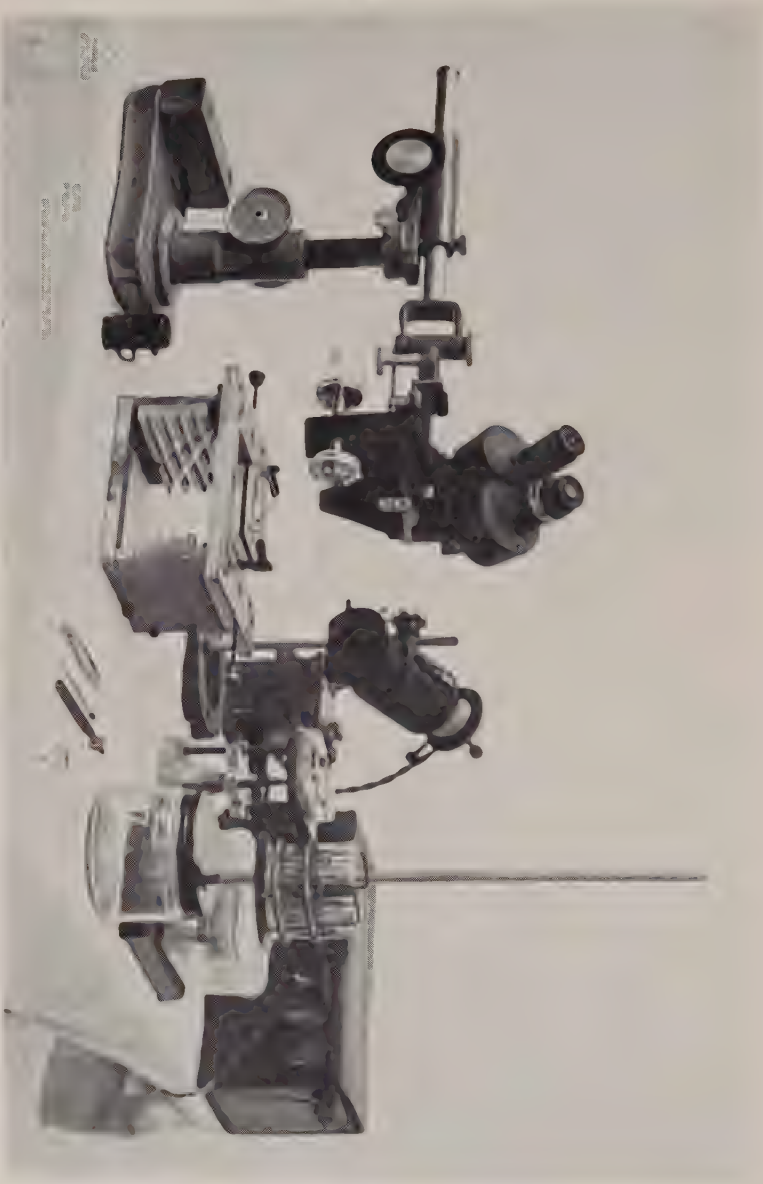

Fig. 7. Modified long-arm microscope and lamp, with dish placed in position on stage. The black press-button switch by stage is connected to an electric counter. *c.* $^{1}/_{50}$ natural size.

PREPARATION OF THE FAUNA FOR MICROSCOPIC EXAMINATION

When the collection tubes from the funnels have to be stored for a period, the catch may deteriorate due to loss of alcohol by evaporation. Addition of glycerol to the collecting solution counteracts this effect to some degree (ARNDT, 1943). ARNDT has also shown that these losses can be further reduced by using waxed corks. This procedure is not considered suitable for tubes containing small, delicate organisms, but the writer has found that sealing the corks with adhesive tape helps to prevent evaporation of the contents. The corks are secured with an opaque, plastic, adhesive tape (Fig. 4 *b*), and the upper exposed surface of the cork is coated with paraffin wax. When the catch is to be prepared for visual examination, the tape is removed, and the sticky adhesive remaining on the glass dissolved with an organic solvent such as acetone.

The equipment required for preparation of the fauna includes a suitable counting receptacle, alcohol reservoir and filtering device. The first is a square or rectangular glass dish with dimensions of 6 × 6 cm ($2\,{}^{3}/_{8}$ in.) and 6 × 3 cm ($1\,{}^{3}/_{16}$ in.) respectively, and 1 cm (${}^{13}/_{32}$ in.) deep (Fig. 4 *c*). The particular size will depend on the nature of the catch; the bigger dish is more suitable for large populations, and where sample debris is present. Except for dimensions, these vessels are similar to the cells used in colorimetric analysis, and are constructed from glass plates cemented together. An automatic pipette (capacity *c.* 25 ml) connected to a reservoir (Fig. 5 *a*) is a useful method to provide a jet of alcohol solution of sufficient force to wash all organisms out of the collection tube. An added convenience is to attach the supply line from the pipette to a hinged, metal boom (Fig. 5 *a*); otherwise it has a habit of falling into prepared dishes and knocking over tubes with disastrous consequences.

The filtering apparatus for removal of excess liquid from the counting vessel, consists of a micro-immersion filter or filter stick (Fig. 4 *d*) with a sintered-glass disk, and is a type used by chemists in micro-analytical work. The pattern is important as it is essential to be able to thoroughly cleanse the filter disk after use, and one with the sintered disk (8 mm *d*) cemented flush with the enlarged head of the glass-tube outlet is particularly convenient from this point of view, e.g. No. K901H of Messrs. Baird and Tatlock (London) Ltd. The filter can be obtained with two porosities (5—40 μ), and for most purposes Porosity 3 (15—40 μ) of the above firm is quite suitable. The filter stick is connected to a filter flask, which in turn is attached to a filter pump or the vacuum side of an air compressor (Fig. 5 *d*). The filter must be cleaned frequently in dichromate solution. The best procedure is to soak it in this fluid for 24 hours, and then wash thoroughly with distilled water. This device is extremely useful in all cases where it is necessary to concentrate a solution containing small organisms.

The contents of the collection tube are carefully washed into a counting dish, with 90 per cent. alcohol from the automatic pipette. This is very important as otherwise small organisms may remain within the tube. For the same reason it is essential to ensure that all glassware is very clean; wide, shallow tubes facilitate this operation. 10—20 ml of alcohol are required for washing, and when completed the dish will contain 12—25 ml of liquid. In order to reduce this volume to a level suitable for microscopic examination, either of two courses may be adopted: the dish may be allowed to stand so that the excess alcohol evaporates, or the process can be hastened by using the filtering arrangement already described. In the latter case the filter stick is carefully washed with a small volume of alcohol when filtering is completed.

To the shallow layer of liquid in the dish is added glycerol in the form of drops to each corner (Figs. 5 *e* and 6); a total of about 28 drops should be sufficient for the larger vessel. The glycerol is allowed to fall from a height of about 5 cm, and when added, care should be taken not to disturb the contents of the dish. The purpose of this operation is threefold:

1. Increasing the viscosity of the liquid makes it much easier to handle and remove organisms, and there is less danger of movement of the dish contents during counting.
2. Glycerol when added to the corners sweeps the contents from the edges towards the centre, thus reducing the numbers present near the corners and sidewalls (Fig. 6). These are the most difficult parts to examine and most counting errors are liable to occur there.
3. When the dish must stand for a period, the presence of glycerol reduces the rate of evaporation of the alcohol, and in this way helps to protect the catch.

QUANTITATIVE AND QUALITATIVE ASSESSMENT OF THE FAUNA

This is the most tedious and time-consuming part of the work as all who have had experience in this field will bear witness. Sample collection is a relatively rapid process, indeed it is quite possible to collect sufficient material over a period of a week to keep a person occupied in quantitative and qualitative evaluation for the rest of the year. On this account considerable attention has been paid to improving methods, and reducing the fatigue associated with long periods of microscopic examination.

A really good binocular microscope with a powerful light source is the first essential if this work is to be carried out accurately and efficiently. The microscope stand used by the writer is a specially constructed modification of a long-arm pattern, manufactured by Messrs. C. Baker of Holborn Ltd., London, to which is attached a binocular body (manufactured by Messrs. Cooke, Troughton and Simms Ltd., York) with 3 turret-mounted objectives, and inclined eyepieces of the wide-field type (Fig. 7). This eyepiece pattern is essential for the comfort of the operator when examination is continued over long periods. The microscope body proper is attached to a stout tube held in a sleeve, and supported by means of a central pillar. It can be moved horizontally with a rack and pinion, and in addition can be rotated horizontally through 360°. To adjust for approximate height there is a rack and pinion on the vertical pillar, which is additional to the normal focusing adjustment on the microscope body. The base of the stand is weighted to keep the instrument stable. It is not possible to give here full details of the modifications of the stand, the principal ones are a longer traverse with a much stouter rack and pinion, and larger diameter knobs for focusing etc. (5.7 cm; 2.25 in. instead of the normal 3.2—3.8 cm; 1.25—1.5 in.), as the latter are too small for comfortable handling over long periods.

The microscope stage (Fig. 7) is a separate unit and consists of a heavy table, larger than normal (20 × 14 cm; 8 × 5½ in.) with a ground-glass plate (11.4 × 10.8 cm; 4.5 × 4.25 in.), and a pair of adjustable spring clips for holding the dish firmly in position. Beneath the dish is placed an opaque, plastic grid with ruled lines. The latter are ruled in one direction only as it is unnecessary, indeed inconvenient, to have the grid ruled in squares. To reduce fatigue and eyestrain during counting, the traversing spaces formed by the lines should be not more than $^2/_3$ of the diameter of the field of view. Plastic grids of different colours — black, blue, green or white — are used, depending on the nature of the fauna. For most purposes black is best, and is essential when large numbers of white or transparent mites are present.

The light source is an 8 V, 6 A 'solid source' filament lamp (Fig. 7) with a movable condenser to provide means for adjusting the size and intensity of the light cone. Only incident or above-stage illumination is used. In addition a polaroid filter is fitted in front of the lamp. Its purpose is twofold: it is well known that if polarized light is directed on to a liquid surface at the polarizing angle, a greater visibility is achieved. In addition the filter subdues awkward reflections arising from the glass sides of the dish. To obtain the best illumination, the lamp should be directed, with an angle of incidence of *c.* 55°, that is the angle formed by the light beam and a line perpendicular to the surface of the liquid. The exact angle can be calculated from the formula:

$$n = \tan a$$

where n is the refractive index of the liquid and a the polarizing angle. In practice the correct angle can be found by adjusting the lamp without filter until the maximum amount of glare is observed when viewed through the microscope. A further improvement is to have the apparatus set up in a darkened cubicle. Owing to the presence of the polaroid filter, which only transmits *c.* 30—40 per cent. of the light source, and the opaque grid — especially the black one — it is essential to have a really powerful lamp; the 48 W lamp described here is regarded as minimal. The writer believes that many of the errors arising in quantitative work are due to insufficient and badly arranged lighting.

The advantages of a square or rectangular counting dish will now be obvious. All the traverses are of equal length, and the sides being truly vertical with right-angled and not rounded corners, make microscopic examination of the edges much easier.

The dish is placed in position on the plastic grid on the microscope stage (Fig. 7), care being taken not to disturb the contents. Then traversing the microscope back and forth across its surface, the organisms are removed as they are encountered, and placed in a glycerol-alcohol mixture in glass squares (Fig. 7). The normal magnification is 35 ×. They are removed with a needle (Fig. 4*f*), which the writer has found far and away the best method for small mites and Collembola. Large mites etc. are taken out with a small spatula or fine forceps (Fig. 4 *e*). The needle is held in the right hand which performs the task of focusing, and procuring and removing the catch, the left hand manipulates the traversing control, and occasionally adjusts the focus with the vertical rack and pinion on the pillar. The liquid in the glass squares consists of glycerol and 90 per cent. alcohol in the proportions 3 : 1. The amount is kept to a minimum, about 2 drops, a practice which renders subsequent handling very much easier. Electric counters are used to keep the main category totals (Fig. 7). Examination of the large dish will take 15 minutes to 4 hours depending on the amount of debris and number of animals present.

The main errors in the counting process are as follows:

1. Organisms present on sides of dish.
2. Organisms missed in traversing process.
3. Organisms inadvertently returned to dish on needle.

To avoid the first, the walls of the dish are initially examined with a low magnification (9 ×), and specimens found are carefully immersed in the liquid. Clean dishes will also greatly assist in reducing the numbers adhering to the sides. The main sources of error in traversing are the two edges parallel to the traversing direction, and to reduce to a minimum these two spaces, viz. the first and last, are each examined 3 times. Lastly owing to the small amount of liquid in the glass squares to which the catch is transferred, there is a danger that an organism does not leave the needle when it is placed in the drop, and hence may be returned to the counting dish. Certain precautions are recommended to prevent this occurrence. The needle consists of a fine entomological pin with the head removed and placed in a special holder (Fig. 4 *f*). It is frequently renewed because after constant use it becomes tarnished and blunt, and in this condition the specimen will not float off so readily. As a further precaution the needle is always placed back in the field of view so that if a specimen has been returned to the dish it can be observed immediately. After practice this operation becomes automatic.

When one is well acquainted with a habitat, it is possible to identify many of the larger specimens during counting; the smaller ones must be mounted on slides and examined with a high-power microscope. The writer's practice is to mount six on a slide, each with a separate, square cover glass (sides of 6 mm; ¼ in.), using lactic-acid mountant. The small cover glass reduces the area to be searched, and as there is a single specimen in each case, it makes it very much easier to keep track of all the material, quite a problem when large numbers are involved. A further advantage is that supports are unnecessary as their weight is such that there is little danger of crushing specimens. It is an advantage to have one end of the slide ground for labelling purposes; for routine identifications, pencil-written details on the ground-glass surface are more convenient than paper labels or a wax pencil. When identification is completed, the specimens are transferred with a fine brush to specimen tubes, 0.8 cm *d* × 5.1 cm *h*($^{5}/_{16}$ × 2 in.; Fig. 4 *g*), containing lactic acid or Oudemans' fluid. This operation requires some practice as the cover glass must first be removed without damage to the specimen. The formula for Oudemans' fluid is as follows:

Alcohol 70%	87 parts
Glacial acetic acid	8 parts
Glycerol	5 parts

The larger organisms in the glass squares are checked again, and then placed in similar tubes. To transfer these, use is made of a needle with a cork disk, slightly larger than the tube diameter, and fixed to the shank of the holder

(Fig. 4 *f*). The needle with specimen on its point is placed in a tube where it is supported by the cork disk. The organism takes a moment to float off, and in the meantime another specimen is secured with a second, similar needle. By the time the latter operation is completed, the first needle is again ready for use.

The tubes are labelled with glass ink, and then taped and waxed (Fig. 4 *g*) in the same way as described for the extraction tubes. A useful accessory is a wire basket (Fig. 4 *g*) to hold 25 tubes. The basket is so constructed that it will fit into a glass jar with a tight-fitting lid (Fig. 4 *a*), a procedure which prevents undue evaporation of the preserving fluid. It is true that traditional storage methods, such as tubes plugged with elder pith or cotton-wool and submerged in alcohol, keep specimens in better condition, but for ecological collections, and where frequent reference and re-examination is necessary, the method described here is more practical. After long periods, some evaporation of the contents is inevitable. A very convenient method for replenishment of tubes without removing the sealed corks is to pierce the latter with a hypodermic syringe, add the required amount of liquid, withdraw the needle and seal with paraffin wax.

ACKNOWLEDGEMENTS

This work has been carried out under a grant from the Forestry Commission. The writer is indebted to Mr. E. J. HOWELL of the Department of Forestry, University of Oxford, who constructed the microscope stage described in the text. The photographs for Figs. 2—7 were taken by Mr. V. STANSFIELD of Rothamsted Experimental Station.

ZUSAMMENFASSUNG

Einzelheiten einer Methode zur Erhaltung ungestörter Bodenproben werden beschrieben, geeignet für den Gebrauch in einem modifizierten Tullgren-Ausleseapparat und besonders zur Anwendung an steinigen Standorten. Ein ungestörter Bodenblock wird aus der Seitenwand eines Profilgrabens gehoben und daraus eine zylindrische Probe von 5,1 cm Durchmesser heraus geschnitten, die in Scheiben von etwa 2 cm Dicke zerlegt wird.

Der Apparat und das Verfahren zur Behandlung der ausgelesenen Fauna (besonders Acarina und Collembola) besteht aus einem viereckigen Glasgefäss (6 × 6 × 1 cm) für die Auszählung, einem Alkohol-Behälter und einem Micro-Immersionsfilter (gesintertes Glas) zur Verminderung des Volumens der Flüssigkeit, in dem sich die Fauna befindet, sowie aus dem Gebrauch von Glyzerin, das zur Erleichterung der mikroskopischen Untersuchung und Behandlung der Organismen bestimmt ist. Ein modifiziertes, langarmiges Mikroskop und ein davon getrennter Tisch werden zur Auszählung und Identifizierung verwendet. Es wird mit auffallendem Licht unter Verwendung eines Polarisationsfilters gearbeitet, der zur Verminderung der Reflexion von den Seiten des Glastellers und zur besseren Sicht in der Flüssigkeit dient. Fehlerquellen während der Auszählung werden besprochen und Einzelheiten der Aufbewahrung des Fanges beschrieben.

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THE IMPORTANCE OF DRINKING WATER TO LARVAL INSECTS

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Many insect larvae have been found to drink water. These include larvae inhabiting stored products, phytophagous species, larvae found in the soil, and species dehydrated during diapause. The water taken in may enable the larvae to withstand unfavourable conditions.

Those who have studied the relations of insects to climatic conditions, and particularly to atmospheric humidity (e.g. UVAROV 1931, BUXTON 1932, MELLANBY 1935, EDNEY 1957) have for the most part stressed the remarkable ability of many of these animals to conserve water and to withstand desiccation, and have paid less attention to the ways in which water is taken into their bodies. Work on this subject is summarized by WIGGLESWORTH in his book "The Principles of Insect Physiology" (1953) where it is shown that drinking of water has often been recorded in adult bees and muscid flies, but not by workers on larval water balance, where the methods by which insects gain water have been studied under the following headings: (i) with the food. (ii) by absorption through the cuticle. (iii) by absorption of water vapour from the air. (iv) as "metabolic water" when food stuffs and food reserves are used. EDNEY, in his recent book "The Water Relations of Terrestrial Arthropods" (1957) quotes LECLERCQ (1946) as reporting drinking in a large number of insects, but adds "the habit is confined to adults".

It is perhaps not surprising that drinking by larval insects has been so largely overlooked. Many seldom or ever drink water, and the ability to withstand dry conditions is widespread and remarkable. Nevertheless, as we will show, a great many larvae can and do drink, and by doing so they affect their metabolism, their life history, their development and their ability to survive exposure to unfavourable conditions. Drinking may not be the most important way in which insects as a whole gain water, but it is more widespread than some of the other processes which have received much greater attention from entomologists.

For the purposes of this paper, it is convenient to consider drinking as it occurs in four different sets of circumstances. We do not believe that the list is exhaustive, and would expect to find drinking in other ecological groups also.

1. INSECTS INFESTING STORED PRODUCTS

The mealworm larva *Tenebrio molitor* L. is well known for its ability to withstand desiccation (BUXTON 1930, MELLANBY 1932) and has often been cited, by one of us as well as by many others, as an insect particularly adapted to living under dry conditions. Yet if given the opportunity, the mealworm larva readily

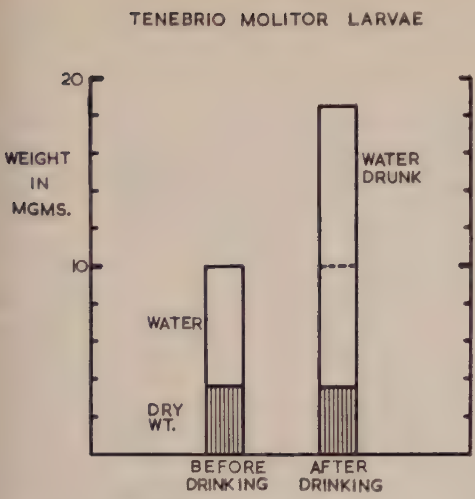

Figure 1. Analysis of mealworm larvae before and after drinking water.

drinks water, and may take in an amount almost equal to its original body weight (Fig. 1). This water is definitely drunk by the mouth and is not absorbed through the cuticle; insects in chill coma in contact with water do not take any up, nor do larvae with their mouths closed with wax. The process of drinking is in fact easily observed and quite unmistakable. If the larva is "thirsty" it seems aware of a drop of water at least a centimetre away, and it will turn out of its course to approach it. During drinking the mouthparts move rapidly, and the mandibles appear to "chew" the surface of the water. Water may be taken up at a rate equal to five per cent of the body weight of the larva per minute, which is as rapid as if a ten stone man (63 Kgs.) drank about six pints (over three litres) in a minute. If a larva is not "thirsty" (a state found soon after drinking to repletion, or when for some reason the water to dry matter ratio in the body is already high), its behaviour is quite different. If its head is put in contact with a drop, the head is rapidly withdrawn, and such a larva can be left for several hours in contact with water without any increase in body weight or of water content.

The water drunk is taken into the gut, and soon afterwards it will be found that the haemolymph, which in a somewhat dehydrated mealworm is very scanty in quantity, has greatly increased in volume (MELLANBY 1958). Incidentally it is interesting to note that a larva which has recently drunk, and has therefore a high proportion of water in its body, looses water by evaporation at very much the same rate as does a more dehydrated specimen. Any slight increase in evaporation would seem to be related to an increase in activity; there is no suggestion that in the drier larva the remaining water is more firmly "bound" by protein or any other substance in the tissues. Thus water which is drunk becomes at once part of the insect's store of that substance.

Drinking of water can be of considerable value to the mealworm. Although this species can live and breed under dry conditions, feeding on bran it can only do so over a comparatively narrow range of temperature, and the growth of the larvae is slow (COTTON & ST. GEORGE 1929, MENUSAN 1936, HOWE & BURGES 1953). When however drinking of water is possible the situation is quite different. Figure 2 shows the rate of growth of mealworms at 25° C and 35° C; all were kept in tubes with bran at 30—35 per cent relative humidity, but some were removed from the bran and given an opportunity to drink once or twice in a week. At 25° C those receiving no water grew slowly, and our results or these conditions agree with those of COTTON & ST. GEORGE (1929) who found a

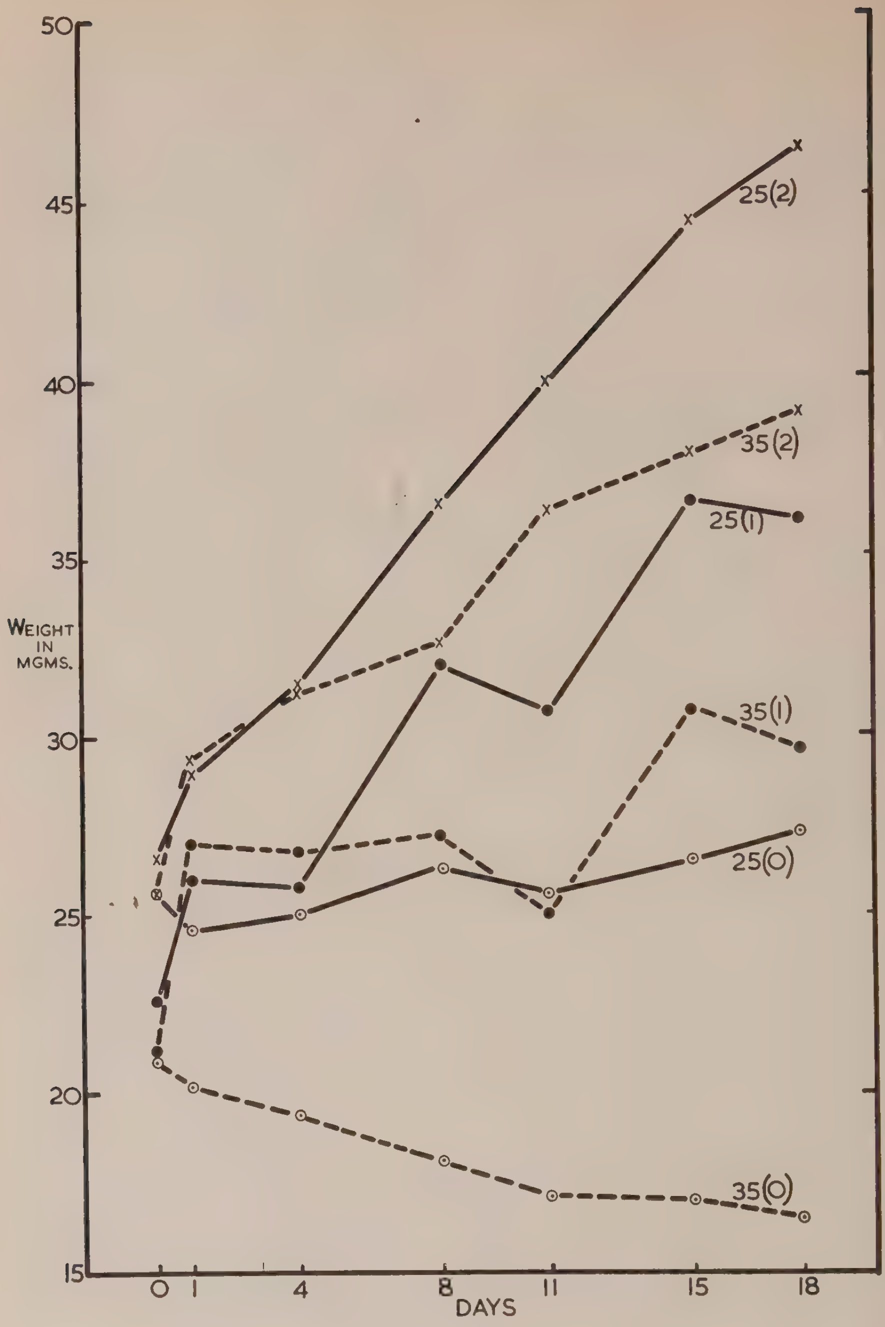

Figure 2. Growth of mealworm larvae at 25° C and 35° C.
0 = no water supplied
1 = access to water once a week
2 = access to water twice a week

"normal" larval period of from 281 to 629 days. When water was provided (it should be noted that the larvae did not drink every time this was possible, and the advantage of giving two opportunities a week was probably that this enabled drinking to take place when it was most advantageous) growth was much more rapid, and larvae could reach their full size within three months. At 35° C the larvae without water lost weight and eventually died. When water was provided, however, they grew nearly as quickly as those kept at the lower temperature.

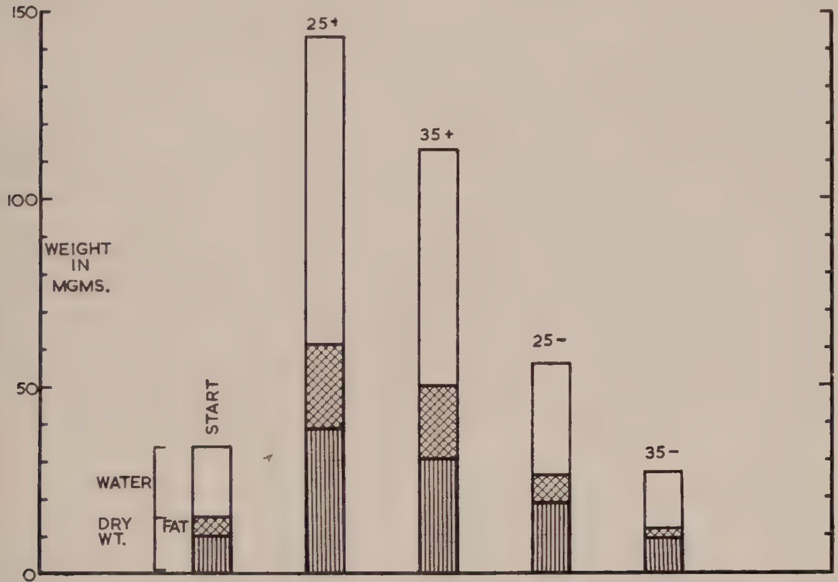

Figure 3. Analysis of mealworms from culture (start) and after one month at 25° C and 35° C with (+) and without (—) water available.

Figure 3 shows the composition of larvae before and after exposure to similar conditions. It will be seen, by comparison with the composition at the beginning of the experiment, that at 25° C the larvae without water, which were exposed for one month in this case, had grown substantially and had increased their fat reserves. Those kept at 25° C with access to water had, however, grown very much more, and there were proportionate increases in their fat reserves and also in their fat-free dry weight. At 35° C without water it is again apparent that the mealworms lost weight, and had, in the one month of this experiment, almost exhausted their fat reserves (that they had used up their reserves suggests that katabolism at any rate had continued normally at this temperature). When water was provided at 35° C the larvae once again were seen to grow well, and to lay down fat reserves. It should be noted that HOWE & BURGES (1953), conclude that "it seems very unlikely that *T. molitor* can complete development when temperatures are continuously over 30° C". Our results support this conclusion where water was not available, and show how drinking may extend the range of this insect.

We do not know how widespread drinking is among larvae infesting stored products. Other species besides *Tenebrio* are able to drink. *Oryzaephilus surinamensis* var. *mercator* Fauv. for instance has been observed to do so, though *Trogoderma granaria* Everts has consistently refused. There are, however, in the literature many accounts of species which live and breed at a higher temperature under high than under low humidities; as this is true of *Tenebrio* by analogy it might be expected that these other species will equally readily drink water if it is available.

Of course drinking can only be useful when there is water available to be drunk. In many food stores this will never happen. In others, particularly when there are considerable fluctuations of temperature, condensation will occur from time to time, and then insects may drink and this may offset the disadvantage of later exposure to high temperature.

In stores of cereals a high humidity may be unfavourable to insects because moulds and mites develop also. With occasional drinking, and the storing in their bodies of the water which is drunk, insects which might otherwise do best at high humidities may well flourish under generally dry conditions.

There are similarities between stored product infestors and desert insects. BUXTON (1924) showed that this latter group could obtain some water by extracting it from apparently "dry" vegetation, but we think that he may have underestimated the importance of dew, which may be formed during the hottest and driest seasons (due to the great variation in day and night temperature) and which if drunk would be stored by the insects which imbibed it.

2. PHYTOPHAGOUS INSECTS

Phytophagous insects feed on food material which contains a great deal of water, and therefore they might be expected not to drink any extra. However many can, and do, drink, and it seems likely that there are many circumstances when drinking is advantageous or even necessary.

Those who have reared caterpillars of butterflies and moths may have often seen them drink, though the habit seems commoner in some species than in others. Thus in " South's Caterpillars of British Moths, Series I" (STOKOE & STOVIN 1948), we find it stated "the partiality of this caterpillar (*Philudoria potatoria*) for a drop of dew has frequently been noted. The old English name of the "Drinker Caterpillar" is therefore an appropriate one". We are not so sure that the name is appropriate in view of the readiness with other species will also drink.

The 'Tomato Moth' (sic) *Diataraxia oleracea* L. is one species which we have found will drink readily. A 300 mgm. larva has been observed to drink 40 mgms. of water in one minute, a speed equivalent to a man drinking two gallons (9 litres) in that time, at a rate of one pint (about half a litre) in three and a half seconds. As in *Tenebrio*, the *Diataraxia* larva when "thirsty" exhibits a positive hygrotaxis, and seems aware of water at least up to a distance of two cms. Their method of drinking seems similar to that found in *Tenebrio*. Larvae of *Diataraxia* which are not " thirsty" behave quite differently. They will walk past a drop of water only a few millimetres away and give no reaction. If the water is directly in their path they walk until they either touch or nearly touch it, then rear up to avoid it and resume their movement in a different direction.

"Thirst" in *Diataraxia* seems to be related to the water content of the larva. The average water content of half grown larvae is in the region of 90 per cent; if it falls much below this level, the larva will drink, and when it rises above 90 per cent drinking stops. This species drinks more rapidly than does *Tenebrio*, but it does not usually take such large amounts in proportion to its body size. This is associated with the fact that *Diataraxia* does not seem to tolerate such large fluctuations in its dry matter to water ratio.

It is not difficult to make *Diataraxia* caterpillars thirsty. As a rule when they are fed on fully turgid cabbage leaves, they will not drink, but if starved under dry conditions they loose water rapidly and will immediately replace this by driking. They are also "thirsty" if fed on wilted leaves with an unduly high dry matter content.

It seems likely that many more caterpillars drink. We have observed that *Pieris brassicae* L. readily drinks, and Mr. Frank SMITH informs us that he has observed several species of noctuid larvae reared in polythene boxes on their food plants drinking drops of water which condense on the surface of the box. Phytophagous beetle larvae have also been found to drink. Thus DOBSON (1955) has shown that the larva of the cabbage stem flea beetle, *Psylliodes chrysocephala* L. will, when desiccated, drink considerable quantities of water.

Although most food plants contain a high proportion of water, larvae living on them still have some risk of having their water balance upset. Consider the case of *Diataraxia* feeding on cabbage. The optimum water content of the half grown larva seems to be about 90 per cent. A turgid cabbage leaf contains only some 85 per cent of water. Provided there is no undue water loss from the larva, its ability to produce faeces containing only 75 per cent of water provides sufficient excess water for its purposes, but if it becomes at all dehydrated, as it does if it falls off its food plant for instance, then a great deal of cabbage must be eaten to restore the balance, for 100 mgs. of cabbage can only provide at most 40 milligrams of water on balance. This amount of food will take at least one hour to consume, whereas the water it yields could be drunk in a quarter of a minute. A perhaps more frequent cause of dehydration is feeding on a wilted leaf. Such a leaf may contain only 70 per cent of water, so the more the larva feeds upon it the more dehydrated does it become. This discussion omits reference to metabolic water, which is of importance in maintaining the water balance of some insects, particularly those which can withstand very dry conditions. Most phytophagous larvae loose water so rapidly that the amount of metabolic water produced in any period must be almost negligable compared with the amount lost by evaporation and excretion. Furthermore any process which increases the amount of metabolic water is liable also to increase the rate at which water is lost, and on balance to increase the likelyhood of dehydration (MELLANBY 1936).

3. LARVAE INHABITING THE SOIL

Insects living in the soil usually find an atmosphere saturated with water vapour, and therefore may not be subject to dehydration. Many feed on roots and stems from which they may obtain ample water. Nevertheless it seems probable that there are many occasions on which soil inhabiting larvae may drink water.

EVANS (1944) showed that wire worm larvae, *Agriotes* sp., have a cuticle which is very permeable to water, and that the water content of their bodies is dependent on the pF value for the water content of the soil. An unfortunate result of this excellent work is that others have, without sufficient evidence, assumed that all soil insects behave in the same way. Some may do so, but the majority seem to have relatively impermeable cuticles, and quite a different mechanism to control their water balance. It is also generally forgotton that Evans in this same paper showed that under certain circumstances *Agriotes* larvae drank water through their mouths.

Some years ago one of us studied the behaviour of the fully fed larvae of the green bottle fly, *Lucilia sericata* Mg. which is to be found in the soil after it has migrated from the carrion on which it has fed (MELLANBY 1938). This larva may have a period of diapause, during which it may become somewhat desiccated. Under these circumstances when the soil in which it lies becomes wet, the water is drunk copiously and is not absorbed through the cuticle. A larva which has been able to drink becomes much larger than one which does not, though both may contain the same amount of dry matter, and the larger larva when it pupates produces a larger sized adult. This difference in size, which affects fecundity to a considerable extent (the larger flies lay larger egg batches) is thus dependent on drinking by the larva.

Phytophagous larvae, such as the wheat bulb fly, *Leptohylemyia coarctata* Fall., and the frit fly, *Oscinella frit* L., migrate through the soil when moving from one host plant (which their attentions may have killed) to another. During this period they may become dehydrated. They do not take up water to offset this through the cuticle, but have been shown in the laboratory to drink readily. The larva of *Opomyza florum* Fabr. which is commonly found during the spring attacking plants of winter wheat in spring has also been found to take up water in a similar way.

4. LARVAE IN DIAPAUSE

As is well known from accounts such as that of HODSON (1937) and LEES (1955) many larvae which have a diapause are to some extent dehydrated during that period, and development is not resumed until the water content is restored to a higher level. It is realised that the termination of diapause, as LEES has described, is a complex phenomenon, and that this increase in water content is only a part, though it may be a very important part, of the whole process.

HODSON mentions several cases where "contact water" is necessary before the diapausing larvae take up water, and he implies that normally this water is absorbed through the cuticle, LEES mentions several cases where water is "imbibed". It seems most probable that drinking is the usual way in which the water content of many larvae in diapause is increased. This is certainly the case with *Lucilia,* as has already been mentioned (see above). *Lucilia* is capable of movement during diapause, and if it is only able to obtain a small quantity of water by drinking, so that its water content is not substantially increased, diapause continues unbroken. Many other larvae (e.g. the European corn borer, *Pyrausta nubilalis* Hbn., BARNES

& HODSON 1956) take up water more rapidly under warm conditions, where drinking would be possible, than at temperatures where they would be in chill coma. As many larvae remain in diapause under dry conditions for considerable periods, it is unlikely that they could survive had they cuticles which are readily permeable to water; a cuticle like the wire worm *Agriotes* allows the insect to absorb contact moisture, but it also means that it cannot survive long in an unsaturated atmosphere without drying up.

DISCUSSION

It seems well established that many insect larvae are able to drink water, and that they may do so under natural conditions. In many cases drinking appears to play an important part in maintaining the water balance. Some of the larvae described drink so readily and efficiently, that it would be difficult to believe that this was not a regular occurrance. None of the larvae studied was observed to drink as rapidly as worker bees which may take nearly their body weight of liquid in a minute (BETTS 1930), but in many the rate of intake is far more rapid than could be achieved by eating or by absorption through the skin. It should perhaps be remembered that bees, though they take in water so rapidly, do so in order to carry it to the hive and do not assimilate it in their bodies.

The importance of drinking must not, however, be overestimated. As has already been stated, there are many insects which seldom if ever drink water, and it is probable that only a minority do so regularly. However in many instances the habit is important, and its possession is one of the qualities which makes insects such an adaptable and successful a group.

ZUSAMMENFASSUNG

Wassertrinken ist bei vielen Insektenlarven festgestellt worden. Die Fälle des Trinkens lassen sich folgendermaßen einteilen:

a. Larven, die von Speicherprodukten leben. Der Mehlwurm, *Tenebrio molitor,* kann unter trockenen Verhältnissen leben, trinkt aber bereitwillig. Nach Tränkung kann diese Art bei höheren Temperaturen leben und heranwachsen als Larven, denen kein Wasser zur Verfügung steht. Wahrscheinlich verhalten sich manche andere Speicherschädlinge ähnlich.
b. Phytophage Larven. Viele decken ihren gesamten Wasserbedarf aus der Nahrung, aber hungernde Individuen oder solche, die von verwelkten Blättern leben, können bereitwillig trinken und so ihren Wasserhaushalt wiederherstellen.
c. Bodeninsekten. Obwohl manche Larven, so z.B. die Drahtwürmer *Agriotes*, Wasser durch ihre Haut absorbieren, kommt auch Trinken vor. Sogar *Agriotes* trinkt unter gewissen Umständen. Andere Larven haben undurchlässige Cuticulae und trinken bereitwillig Wasser, wenn es erforderlich ist, ihr Wassergleichgewicht wiederherzustellen.
d. Diapausierende Larven. Viele Larven haben während der Diapause einen niedrigen Wassergehalt, und bevor dieser nicht ansteigt, kommt die Diapause normalerweise nicht zum Abschluß. Bei Larven, die während der Diapause bewegungsfähig sind, kann das Wassertrinken eine Vorbedingung für die Beendigung der Diapause sein.

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SOME EXPERIMENTAL EVIDENCE FOR THE SURVIVAL VALUE OF THE ROOTPIERCING HABITS OF *MANSONIA* LARVAE (CULICIDAE) TO PREDATORS

BY

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Mansonia larvae pierce *Pistia* plants with their breathing syphons, and are much less motile than most mosquito larvae. In clear water with no plants present they are eaten as readily by predators (water bugs, beetle larvae, fish) as *Culex fatigans*. If the *Mansonia* are attached to *Pistia* they are less frequently eaten.

The larval instars of *Mansonia* species (the first instar probably excepted) exhibit a peculiar siphonal oxygen uptake: the siphon pierces living vegetable tissue and connects with an air cavity inside the host plant. A root-piercing habit is also recorded for representatives of some other Culicidae genera (*Aedomyia*, *Ficalbia*). In the genus *Mansonia* however, the parallel siphonal modifications are most developed, as they have a saw-edged apparatus provided with hooks, adapted for piercing and anchoring.

In a *Mansonia* breeding place investigated (Sentani lake, Neth. New Guinea), the larvae occur in eutrophic waters, where they were observed to lead an almost sessile life under cover of the roots of the host plants, mainly the common water lettuce, *Pistia stratiotes.* This is a floating water-weed with long, pendulous roots and shorter rosette roots, the latter close to the surface. *Mansonia* larvae were found in large numbers here, as well as another root-piercing species: *Ficalbia modesta.* Predators were present in great numbers, but surface dwelling, free swimming mosquito larvae were scarce — the minute *Ficalbia minima* excepted — while food and space in which to live seemed to be present abundantly.

It seemed likely that the immobility of *Mansonia* (and *Ficalbia*) larvae is an advantageous device against predators which mainly react to distinctly moving prey. It should be noted that this immobility never can be complete. A *Mansonia* larva has to perform a constant fanning movement with the mouthbrushes for its food uptake— a movement which immediately stops for some time when the animal is disturbed by a passing object. Other movements have to be performed at moulting, and at pupation.

Mansonia certainly belongs to the most abundant and most noxious mosquito species in many parts of New Guinea (and in many other parts of the world); some species are of medical importance as vectors of filariasis.

The aim of the experiments reported in this paper was to compare two larval types, the free swimming and the sedentary, in the risk they run from some local predators under test conditions. Free swimming species could not be collected from the *Mansonia* breeding place in sufficient numbers for the tests, and *Culex fatigans* larvae were used. This substitution does not seem unreasonable as the behaviour of *Culex fatigans* does not differ substantially from a *Culex* species taken occasionally from the *Pistia* field.

MATERIAL

Fourth instar larvae of *Mansonia uniformis* and *M. bonnewepsterae* were collected from a breeding place in the Sentani lake, described in more details elsewhere (v. D. ASSEM, 1958). As these larvae are very much alike in dimensions and breathing habits, they were used indiscriminately in all experiments. Fourth instar *Culex fatigans* larvae, collected from dirty drums, were used as free swimming species. Predators were collected in the *Mansonia* breeding place together with the Diptera-larvae.

Predators used in the tests were :

Diplonychus sp. (Belostomatidae). Adult, free swimming bugs, about 2 cm long; the hind legs are modified for rapid swimming, the front legs curved and spine like for catching prey.

Cybister sp. (Dytiscidae). Slender, mobile larvae, about 6 cm long.

Eleotris sp. (Gobiidae). Only young specimens, 2—3.5 cm long, of this fish were collected. This species is not very mobile, most times lurking for food from under cover.

The majority of tests were done with *Diplonychus* and *Eleotris*. The host plant used was *Pistia stratiotes. Mansonia* larvae readily accept this plant; there is no preference for a special location on the roots. All experiments were carried out in small glass tanks, 20—15—12.5 cm in dimensions.

TECHNIQUE AND RESULTS

M. uniformis/bonnewepsterae larvae and *C. fatigans* larvae of about the same dimensions were offered simultaniously to a predator. Tests were done in tanks containing clear water only, parallel ones in tanks containing *Pistia* plants. In a *Pistia* tank the predator was introduced half an hour after the introduction of the larvae to be sure that all *Mansonia* were attached by that time. At the end of every individual test, which lasted about 7 hours for *Pistia* tanks and about half that time for clear-water tanks, the numbers left of either mosquito species were counted. Predators were kept without food for one day before they were tested; after a test they were offered larvae to test the appetite— which was positive after all *Pistia* tests.

No attempt was made to imitate the natural conditions of the breeding place entirely or to work with concentrations of predator or prey comparable to natural conditions. Predators had to be confined to the small space of the test tanks; in nature the larval density varies considerably, the roots generally are far more dense. The *Pistia* roots in the breeding place were found covered with debris, producing an extra cover for the larvae. Light conditions were different in nature; in the tests light from the side — shielded as far as possible — could produce some extra contrast favouring the predators.

Table I. *Clear water tests. Larval density 10M—10C.*

predator	numb. tests	numb. larv. offered		numb. larv. left		tot. time tests in hours	mean numb. prey/hour testing		ratio
		M	C	M	C		M	C	M/C
Diplonychus	10	100	100	22	44	37.30	2.34	1.76	1/0.75
Eleotris	11	110	110	44	44	57.45	1.13	1.13	1/1
Cybister	3	30	30	22	16	10.00			

The results of the tests with larval density 10 *Mansonia*- 10 *Culex* are summarized in Table I (clear water) and Table II (*Pistia*).

In clear-water tanks the number of larvae caught is rougly the same for both mosquito species. The slightly higher proportion of *Mansonia*'s caught by *Diplonychus* sp. is most probably due to the attraction of a contrasting object to detached *Mansonia*. The larvae approach such object and try to attach themselves to it by fierce backward movements. Some *Mansonia* were observed trying to establish themselves on the floating dark-brown bugs, subsequently to be caught by this predator. In the far less conspicuous and bottom dwelling *Eleotris, Culex* and *Mansonia* larvae suffered alike from predation.

Table II. Pistia *tests. Larval density 10M—10C.*

predator	numb. tests	numb. larv. offered		numb. larv. left		tot. time tests in hours	mean numb. prey/hour testing		ratio
		M	C	M	C		M	C	M/C
Diplonychus	11	110	110	103	68	101.00	0.07	0.41	1/6
Eleotris	10	100	100	98	29	65.45	0.03	1.07	1/35
Cybister	2	20	20	19	10	15.15			

In the *Pistia* tanks the predation on the same larval density is lower, due to a general protection from the weeds. At the same time, however, the relative numbers of larvae caught by predators differ substantially for the two mosquito species: the ratio *Mansonia-Culex* preys being in total about 1 to 10. This difference is likely to be due to an extra protection, derived from the root-piercing habits, for the *Mansonia* species.

Table III. Pistia *tests. Larval density 30M—10C.*

predator	tests numb.	numb. larv. offered		numb. larv. left		tot. time tests in hours	mean numb. prey/hour testing		ratio
		M	C	M	C		M	C	M/C
Diplonychus	7	210	70	193	53	53.15	0.3	0.3	1/1

One test series was arranged for *Diplonychus* sp. with increased larval density: 30 *Mansonia*- 10 *Culex* per tank. The increase of the number of available *Mansonia's*, increases the number of *Mansonia* preys, bringing the ratio M-C to about 1 to 1. The results of this series are summarized in Table III.

Table IV. Pistia *tests.* Mansonia *only, larval density 10 cq. 30.*

predator	numb. tests	numb. larv. offered	numb. larv. left	tot. time tests in hours	mean numb. prey/hour testing	
Diplonychus	10	100	94	70.45	larval dens. 10	0.08
Diplonychus	4	120	105	31.00	larval dens. 30	0.4

In a series of *Pistia* tests, *Culex fatigans* larvae were omitted as a deterrent prey. The results from these tests differ for *Diplonychus* and *Eleotris.*

For *Diplonychus* the numbers of *Mansonia* larvae taken are comparable with the numbers found in the simultaneous *Mansonia-Culex* tests for both 10 c.q. 30 *Mansonia* larvae per tank (Table IV). In the absence of a deterrent prey *Eleotris* sp. however, takes a higher number of *Mansonia* than in the choice tests (Table V), but the score is lower than that for free swimming *Culex* larvae in *Pistia* tests (cf. Table II).

Table V. Pistia *tests.* Mansonia *only, larval density 10.*

predator	numb. tests	numb. larv. offered	numb. larv. left	tot. time tests in hours	mean numb. prey/hour testing
Eleotris	14	140	73	88.00	0.7

Conclusions

In the absence of a suitable host plant *Mansonia* and *Culex* larvae suffer alike from the predators tested. In tanks containing *Pistia* plants, *Mansonia* larvae suffer considerably less from predation than free swimming larvae. At a higher *Mansonia* density an increase in predation was recorded. In tests without alternative prey the numbers taken by *Diplonychus* were comparable with those taken in choice tests; the numbers taken by *Eleotris* were considerable higher. In the breeding place however a number of alternative prey is always present, and it is doubtful whether predation by this fish is of any practical importance.

The tests produce some evidence for the advantage of the root-piercing habits of *Mansonia* in relation to predators. This habit, which is acquired independantly a few times in the Culicidae family, is an adaptation reducing predation.

This result is in general agreement with the conclusions of Jackson (1953). She found that the predatory mosquito larvae *Culex* (*Lutzia*) *tigripes*, if offered several types of mosquito larvae as prey, ate the greatest number of the most active species. Thus *Aedes aegypti*, which is very active, was eaten more often than *Culex sp.*, and *Culex* more often than the comparatively inactive *Anopheles gambiae.* It seems likely that *Mansonia* would be eaten by *Lutzia* even less frequently than *Anopheles.*

ZUSAMMENFASSUNG

Mansonia-Larven besitzen Atemsiphonen, die geeignet sind, unter Wasser Pflanzengewebe anzubohren (besonders *Pistia stratiotes*). Die Larven atmen Luft, die sie aus den Pflanzen beziehen. Diese Larven sind verhältnismäßig unbeweglich.

Mansonia- und *Culex fatigans*-Larven sind den Nachstellungen folgender Räuber ausgesetzt: Erwachsenen Wanzen von *Diplonychus* sp., Larven der Käfergattung *Cybister*, Jungfischen der Gattung *Eleotris* (Gobiidae).

Im klarem, pflanzenfreien Wasser werden beide Arten Mückenlarven in annähernd gleicher Anzahl gefressen. Das zeigt, daß *Mansonia* nicht geschmackswidrig ist.

Wenn die *Mansonia* an *Pistia* angeheftet sind, werden sie in viel geringerer Anzahl gefressen als *Culex*, vorausgesetzt, daß beide Arten vorhanden sind. Wenn keine *Culex* zur Verfügung stehen, fressen *Diplonychus* und *Cybister* nur wenige der angehefteten *Mansonia*, während *Eleotris* seine Nachstellungen verstärkt.

Daraus wird gefolgert, daß die durch diese Lebensweise gewonnene Unbeweglichkeit für *Mansonia* vorteilhaft ist und eine der Ursachen darstellen könnte, daß sie in Neu Guinea so häufig ist.

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THE MODE OF ACTION OF AN ORGANOPHOSPHORUS INSECTICIDE (DDVP)

SOME EXPERIMENTS AND A THEORETICAL DISCUSSION

BY

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In the course of our investigations on the mode of action of organophosphorus insecticides, we met with an insecticide which combines an extremely high toxicity to houseflies with a fairly low toxicity to mammals (VAN ASPEREN, 1957a). This insecticide, DDVP (= 0,0-dimethyl 0-2,2 dichlorovinyl phosphate) proved to be highly active in the vapour state and it could be shown that DDVP vapour concentrations which caused death of houseflies within two minutes were quite harmless to mice, even when these were exposed for as long as 6 hours. Large differences in susceptibility were also found when DDVP-solutions were injected into mice (subcutaneously or intravenously and into houseflies (intrathoracically). The results are shown in Table I.

Since many investigators claim that organophosphorus compounds exert their action by the inhibition of cholinesterase activity in the nervous system we studied the process of cholinesterase inhibition by DDVP in vitro. For comparison we also studied the aliesterase activity in houseflies and mouse brain.

CHOLINESTERASE INHIBITION AND TOXICITY

Method. The cholinesterase activity was determined manometrically by the Warburg method at pH 7.4 (bicarbonate-CO_2 buffer) and 37° C. Enzyme preparations were obtained, *a.* by dilution of horse serum, *b.* by homogenizing mouse brain tissue with saline in a small type Waring blendor, and *c.* by a similar treatment of houseflies (*Musca domestica*) or housefly heads. The total volume of the reaction mixture in the Warburg vessels was 2.2 ml, consisting of 1 ml of the enzyme preparation, 1 ml of inhibitor solution and 0.2 ml of substrate solution. The aliesterase activity was determined by the same method using methylbutyrate or tributyrin as substrates. As the dose of DDVP required for 50% inhibition (I_{50}) is, at least in many cases, greatly dependent on the enzyme concentration, all inhibition values were determined or calculated with respect to a control activity (activity without inhibitor) corresponding with approximately 60 μl CO_2/30 min./ml of reaction mixture.

In the experimental conditions described above the I_{50} of DDVP for the cholinesterase of horse serum and mouse brain proved to be about 10^{-7} M., whereas housefly-cholinesterase was 50% inhibited by only 10^{-9} M. The results of our determinations are shown in Table I, which allows of an easy comparison with the LD_{50}-values.

The conclusion seems justified that toxic action and inhibition of cholinesterase by DDVP are causally related. It should not be overlooked, however, that the remarkable correlation between the susceptibility of the cholinesterase to the inhibitory action and the susceptibility of the organism as a whole to the toxic action of DDVP could just as well be caused by some common factor. If for instance DDVP were metabolized by mammalian tissues and homogenates much faster than by those of houseflies, this would result in much lower DDVP concentrations in the mice as compared with the flies and this would give rise both to low enzyme inhibition and to low toxicity, even though these had no direct causal relationship at all. Such doubts as to the correctness of the theory, according to which organophosphorus insecticides exert their action by cholinesterase-inhibition are, in our view, quite justified. According to our own experiments (VAN ASPEREN 1957b, 1958) and to many data in the literature (SPENCER & O'BRIEN, 1957), the problem of the mode of action of organophosphorus compounds should remain open for discussion at present.

TABLE I.

Toxicity and enzyme inhibition
ChE = cholinesterase; AliE = aliesterase

DDVP-LD_{50}		DDVP-I_{50}	
Mouse intravenous inj.	10 μg/gram	Mouse brain-ChE	1×10^{-7} M.
„ subcutaneous inj.	15 „	„ -AliE	1.5×10^{-7} M.
		Horse serum-ChE	1.5×10^{-7} M.
Housefly injection	0.18 μg/gram	Housefly-ChE	1.5×10^{-9} M.
„ topical applic.	1.5 „	„ -AliE	1×10^{-9} M.

Different susceptibilities of the homogenates to inhibition by DDVP need not necessarily mean that the enzymes are chemically different. Other possibilities exist as well, e.g. other compounds such as proteins, present in the suspensions, might influence the inhibition values by binding and thus inactivating the DDVP molecules. It is the purpose of this paper to describe some investigations into the problem as to whether there do actually exist significant differences between the cholinesterases of houseflies and mice.

SUBSTRATE SPECIFICITY OF THE HOUSEFLY-CHOLINESTERASE

In order to investigate the substrate specificity of the housefly-cholinesterase the following experiments were undertaken. Fly head homogenates were combined with several concentrations of a number of different substrates, viz. acetylcholine, acetyl β-methyl choline, butyrylcholine, benzoylcholine, triacetin, o-nitrophenylacetate, phenylacetate, n-amylacetate, methylacetate, ethylacetate, methylbutyrate and tributyrin. If a substrate was found to be hydrolysed, further experiments were performed to find out to what extent, if any, the hydrolysis had to be ascribed to the action of the cholinesterase, i.e. the enzyme splitting acetylcholine. In this way a fairly clear picture of the hydrolysing capacities of the cholinesterase was obtained. The details of this study will be published elsewhere, but the most important results concerning the cholinesterase are shown in fig. 1. From these it appears that the fly head-cholinesterase in general shows the characteristic properties of the "true cholinesterase" as found in mammalian brain tissue (AUGUSTINSSON,

1950) viz: inhibition by excess substrate as far as choline esters are concerned, high activity against triacetin and nearly no activity against benzoylcholine. The only deviation from the main pattern of mammalian brain-cholinesterase is the

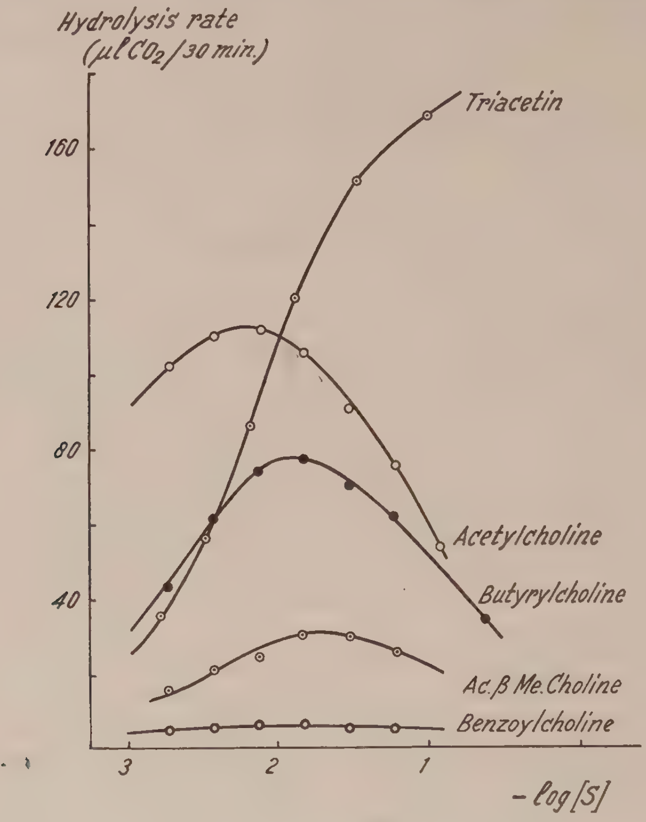

Fig. 1. Substrate specificity of fly head cholinesterase

rather rapid hydrolysis of butyrylcholine, which, however, showed the well known inhibition by excess substrate in accordance with findings of Wolfe & Smallman (1956) and contrary to the data given by Metcalf, March & Maxon (1955).

It may be concluded that there most probably exist specific differences between the enzymes. There is, however, no reason to consider the housefly-cholinesterase as a special type of cholinesterase and so far, large chemical differences between mouse brain and housefly cholinesterase are not to be expected.

Combination experiments

These experiments were performed in order to decide whether the differences in inhibition by DDVP between mouse brain and fly head homogenates are due to structural differences between the cholinesterases themselves or to other factors present in the homogenates. The following technique was used.

Homogenates of mouse brain (M) and fly head (F) were prepared. The cholinesterase activity was measured with 1.5×10^{-2} M. acetylcholine as substrate and the inhibition by 10^{-9}, 10^{-8} and 10^{-7} M. DDVP was determined. It was found, in accordance with the I_{50}-values reported in Table I, that a concentration of 10^{-8} M. DDVP caused 100% inhibition of the cholinesterase-activity in the fly head suspensions and about 10% inhibition in the mouse brain homogenates. Mixtures of the homogenates were then prepared ($MF = 1$ part of $M + 1$ part of F; $MF_3 = 1$ part of $M + 3$ parts of F; $MF_9 = 1$ part of $M + 9$ parts of F) and combined with 10^{-8} M. DDVP. The inhibition values actually obtained were then compared with those calculated according to two hypothetical models, one of which is representative for the first, the other for the second possibility mentioned above. These hypothetical models will be dealt with in full detail below.

Hypothesis 1: That no essential difference exists between the cholinesterases of the two homogenates, the difference in inhibition being caused by the binding of DDVP-molecules to other (most probably protein) molecules. The occurrence of a large excess of other inhibitor-binding protein molecules in the homogenates of mouse brain (thus necessitating the use of much larger amounts of inhibitor to obtain equal inhibition as compared with fly head homogenates) is suggested by an observation of COHEN & WARRINGA (1953). These authors found that ox red cell suspensions contain large amounts of DFP-binding proteins, which considerably increased the concentration of DFP required for 50% inhibition. Similar conditions could exist in the mouse brain homogenates with respect to DDVP. If this hypothesis is correct, mixtures of M and F will contain large amounts of DDVP-binding molecules which will influence the inhibition both of the fly head cholinesterase and of the mouse brain cholinesterase. Two further assumptions will be made: *a.* Inhibition by DDVP is assumed to be practically irreversible (This means hat the number of molecules occupied by DDVP-molecules is equal to the number of DDVP-molecules added, at least if the reaction time is sufficiently long). *b.* The affinity of DDVP for the other DDVP-binding substances is assumed to be equal to that for the cholinesterase. If these assumptions are not made, the calculation will be much more complicated, though the results, in our view, will not be strikingly different from those obtained below.

Calculation: The I_{50} for mouse brain is approximately 100 times that for fly heads. This means that the total concentration of DDVP-binding molecules is 100 times the concentration of DDVP-binding molecules in the fly head homogenates. This latter concentration may be taken to be a (mol./ml). The inhibition caused by a DDVP-concentration p (mol./ml) will be $\frac{p}{a} \times 100\%$ in the case of the fly head homogenates (or 100% if $p > a$) and $\frac{p}{100a} \times 100\% = \frac{p}{a}\ \%$ in the case of the mouse brain homogenate (assuming $p < 100a$). The mixture MF will contain $\frac{101a}{2}$ DDVP-binding mol./ml and an inhibitor dose p will cause $\frac{2p}{101a} \times 100\%$ $= 1.98 \times \frac{p}{a}\ \%$, which means that the inhibition will be approximately twice that obtained with the mouse brain suspension and the same dose of DDVP. In a similar way the inhibition values may be calculated for the mixtures MF_3 and MF_9.

If the percentage inhibition of mouse brain homogenate caused by a certain dose is known it will thus be possible to calculate the inhibition values which according to *hypothesis 1* would be expected if the same dose is applied to mixtures of mouse brain and fly head homogenates.

Hypothesis 2: That the difference in susceptibility to inhibition by DDVP is caused by differences between the enzymes themselves. If no further information on the nature of the difference is available, it is difficult to predict the inhibition values which according to this hypothesis may be expected to occur in the combination experiments.

If, for instance, the enzymes of fly heads and of mouse brain only differed by their turnover numbers, calculation could result in inhibition values which are equal to those calculated according to hypothesis 1. In this case the combination experiment is unable to distinguish between this possibility and that described in the first hypothesis. For biochemical reasons, however, this possibility is rather unlikely.

If the second hypothesis is accepted it is much more reasonable to assume that in our combination experiments the two components of the mixture (M and F) behave independently. This possibility is clearly distinct from that described in the first hypothesis and will be used as the basis for our calculation.

Calculation: Suppose we use, in our experiment, a DDVP-concentration giving a% inhibition of the fly head homogenate and b% inhibition of the mouse brain homogenate. When mixtures of homogenates, showing equal enzyme activity, are exposed to the same DDVP-concentration, the inhibition will be $\frac{a+b}{2}$ % for MF, $\frac{3a+b}{4}$ % for MF_3 and $\frac{9a+b}{10}$ % for MF_9. These calculations need some correction if the homogenates M and F show different activities in the absence of inhibitor, as they do in our experiments.

Although it cannot be maintained that the two hypothetical models dealt with above are the only possible ones, we nevertheless believe that they are more or less representative for the two, physiologically the most probable, ways of explaining the striking difference in susceptibility to inhibition by DDVP between mouse brain and fly head homogenates.

The results (Table II) show clearly that there is nearly complete agreement between the inhibition values actually obtained and those calculated according to hypothesis 2. Very probably, therefore, the differences in inhibition will, at least partly, be due to differences between the enzymes themselves and not only to differences in other factors or compounds present in the homogenates.

THE NATURE OF THE DIFFERENCE

Valuable evidence as to the nature of the difference between the two enzymes was gained from the fact that in the case of mouse brain homogenates a slow decrease of inhibition could be observed during the first hours after the addition of the substrate. No such decrease, however, was observed in the case of fly head homogenates. It was found that 50% reactivation of mouse brain-cholinesterase activity was obtained in the course of two hours. The reactivation rate is a little less than that found by ALDRIDGE & DAVISON (1953) for dimethylphosphate-inhibitors and rabbit erythrocyte-cholinesterase. It is, therefore, quite reasonable to consider the inhibition of mouse brain-cholinesterase as slowly reversible, whereas that of fly head-cholinesterase is completely irreversible. A further quantitative analysis of the inhibition kinetics will be given in another paper (VAN ASPEREN & DEKHUIJZEN, 1958). So far it seems justifiable to conclude that the difference in susceptibility to DDVP-inhibition is partly due to this difference in reversibility.

TABLE II.

Inhibition of homogenate mixtures by DDVP

M = Mouse brain homogenate, conc. 9 mg of tissue/ml; F = Fly head homogenate, conc. 1.25 heads/ml. The Warburg vessels contained 2 ml of homogenate (main compartment) and 0.2 ml of 3% acetylcholinehydrochloride (side arm). The final substrate concentration was 1.5×10^{-2} M. The homogenate was incubated for 1.5 hours with DDVP before addition of substrate. The table shows *first* the activity (act.) in mm³ CO_2/30 min. at 37° C of the uninhibited control (DDVP-conc. = 0), *then* the inhibition (inh.) obtained with the DDVP-conc. (Mol.) given in the head of the column.

	M			F			MF		MF_3		MF_9	
DDVP-conc.	0 act.	10^{-8} inh.	10^{-7} inh.	0 act.	10^{-9} inh.	10^{-8} inh.	0 act.	10^{-8} inh.	0 act.	10^{-8} inh.	0 act.	10^{-8} inh.
Exp. I.	114	11%	65%	117	73%	101%	120	61%	123	84%	117	96%
	calculated according to hypothesis 1 :							22%		43%		100%
	” ” ” ” 2 :							56%		78%		92%
Exp. II.	111	10%	61%	143	54%	101%	132	61%	138	83%	142	94%
	calculated according to hypothesis 1 :							20%		38%		89%
	” ” ” ” 2 :							61%		81%		93%

DISCUSSION

It is generally accepted that in mammals intoxication by organophosphorus compounds is caused by inhibition of the cholinesterase-activity in the nervous system, although other mechanisms of action are not wholly excluded (see for example SPENCER & O'BRIEN, 1957). It seems worth while to discuss our results, especially with regard to the problem as to whether there is a causal relationship between cholinesterase-inhibition and toxic action of DDVP in insects.

The striking correlation between the LD_{50} - and I_{50} - values as shown in Table I does not necessarily point to cholinesterase-inhibition as the cause of intoxication in insects. Such a correlation could also be expected if, for instance, the DDVP-concentrations are considerably decreased by some factor acting both in the living mouse and in the mouse brain homogenates. Our combination experiments, however, failed to demonstrate the presence of such a factor in mouse brain homogenates. On the contrary they strongly favoured the view that the differences in susceptibility of the cholinesterases to DDVP-inhibition are largely due to differences between the enzymes themselves. It is difficult to explain a correlation of enzyme properties and susceptibility to DDVP-intoxication, unless some direct causal relation is assumed. Our experiments do therefore provide some evidence in favour of the "anti-cholinesterase-theory" of intoxication.

In a previous publication (VAN ASPEREN, 1957b), however, evidence was furnished against the essential role of cholinesterase-inhibition in DDVP-intoxication. Moreover, the data of Table I show that a similar marked difference was found when the susceptibility to DDVP of the aliesterase activity in the homogenates was studied. So, many of the arguments supporting the "anti-cholinesterase-theory" of DDVP-intoxication also apply to a possible "anti-aliesterase-theory" or a more general "anti-esterase-theory".

So far we can only conclude that the evidence concerning the relation between cholinesterase-inhibition and DDVP-intoxication is still conflicting, though many of the experiments described in this paper favour the view that there actually exists a causal relation. It is expected that a more detailed study of the aliesterase and its physiological function in the insect, which is underway at the moment, will throw some more light on this problem.

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ZUSAMMENFASSUNG

1. Es konnte gezeigt werden, dass die Giftigkeit und besonders die Dampfgiftigkeit von DDVP (0,0-Dimethyl 0-2,2 Dichlorvinyl-Phosphat) gegenüber Stubenfliegen (*Musca domestica*) sehr viel grösser ist als gegenüber Mäusen. Dieser Unterschied in der Giftwirkung ist auffällig mit einem Unterschied in der Hemmbarkeit der Cholinesterasen in homogenisierten Geweben korreliert (siehe Tabelle I).
2. Der Unterschied in der Hemmbarkeit wurde eingehend in der Absicht untersucht, nachzuforschen, ob es sich hier um eine Differenz zwischen den Enzymen selbst oder um einen Unterschied bei anderen in den Homogenaten anwesenden Substanzen oder Faktoren handelt. Man könnte dabei an die Anwesenheit bestimmter Eiweissmoleküle denken, die DDVP binden und dadurch inaktivieren. Das Ergebnis unserer Untersuchungen zeigt, dass die Enzyme selber auffallende Unterschiede aufweisen, die höchstwahrscheinlich zu einem sehr beträchtlichen Teil für den Unterschied in der Hemmbarkeit verantwortlich gemacht werden können.
3. Obwohl die Hemmungsreaktionen sehr verschieden sind, unterscheidet sich die Fliegen-Cholinesterase in ihrer Substratspezifizität nicht wesentlich von der sogenannten „true cholinesterase", die im Gehirn der Saügetiere gefunden wird.
4. Die Bedeutung unserer Ergebnisse wird speziell in Beziehung auf die Frage diskutiert, welche Rolle die Cholinesterase-Hemmung bei der Vergiftung durch DDVP spielt. Da gefunden wurde, dass auch die Ali-Esterase-Aktivität (Substrate: Methylbutyrat und Tributyrin) der Fliegen in vitro viel stärker von DDVP gehemmt wird als diejenige des Mäusegehirns, wird in dieser Diskussion auch die Möglichkeit einer Ali-Esterase-Hemmung als Ursache der Vergiftung hervorgehoben.

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SOME INSECTARY EXPERIMENTS WITH PREDACIOUS MITES TO DETERMINE THEIR EFFECT ON THE DEVELOPMENT OF *METATETRANYCHUS ULMI* (KOCH) POPULATIONS

BY

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As field spraying experiments give results which are difficult to resolve in terms of individual predator species, a series of experiments has been carried out in an insectary to find out what effect predacious mites of various species have upon populations of *Metatetranychus ulmi* (Koch), in the absence of other prey. Known numbers of each mite species were placed on seedling plants, and the development of the resulting populations followed.

In one experiment, where initial numbers of 5, 25 or 50 *M. ulmi* females were placed on a plant, together with five females of *Typhlodromus tiliae* Oudms., the *M. ulmi* population in every case remained throughout the following three months at a density of less than one mite per leaf, which was less than a total of 50 mites per plant. Where the same numbers of *M. ulmi* started in the absence of *T. tiliae*, a rapid increase of *M. ulmi* resulted in populations of over 3000 mites per plant in up to eleven weeks.

It was also found that both the size of the host plant, and the initial ratio of *M. ulmi* and *T. tiliae*, had an effect on the development of the *T. tiliae* populations.

Of other predacious species, *T. finlandicus* was similar to *T. tiliae* in its effect on *M. ulmi*. Although *T. umbraticus* itself increased steadily, it exerted little effect on the *M. ulmi* population, nor did *T. tiliarum* which, however, did not develop well under these conditions. *Phytoseius macropilis* (Banks) was also slow to increase and had little effect on the *M. ulmi* population.

Field experiments carried out in England and other parts of the world have repeatedly shown an inverse relationship between levels of the Fruit Tree Red Spider Mite (*Metatetranychus ulmi*) and various *Typhlodromus* species (e.g. GILLIATT, 1935; COLLYER & KIRBY, 1955 and 1958; MATHYS, 1955; CLANCY & MCALISTER, 1956). As *Typhlodromus* mites are partially predacious in habit and feed readily on *M. ulmi* this has led to the fairly general assumption that they are an important factor in the natural control complex associated with this mite. It has however been pointed out by critics of this theory that field trials are invariably complicated by chemical applications, whose side effects are not known, and the presence of insect predators which in some cases may have been overlooked or ignored. HUFFAKER & KENNETT (1953), in their experiments on the control of *Tarsonemus pallidus* Banks by *Typhlodromus reticulatus* Oudms. on strawberries, realised the importance of ensuring that their results were due to differing *Typhlodromus* levels, and not to some chemical side effect. They took plants into the greenhouse already infected with both species of mites, and by repeated hand removal of *Typhlodromus* from one group of plants caused increases of

T. pallidus populations similar to those where parathion was used in the field to eliminate *Typhlodromus.*

In orchards where a correlation between levels of *Typhlodromus* and *M. ulmi* has been observed, the differences in *Typhlodromus* populations have invariably been caused by chemicals. It is difficult to obtain such differences in an orchard by other means, so a number of experiments have been made on small plants in an insectary, where both spray applications and insect predators were avoided, and the initial levels of both predacious and phytophagous mites were chosen. In English commercial orchards *M. ulmi* is the main phytophagous mite present, and *Typhlodromus tiliae* Oudms. the only predacious mite of importance, so most attention has been paid to the interaction of these two species.

EXPERIMENTAL PROCEDURE

Because of their susceptibility to mildew, apple seedlings as used by MASSEE (1947) failed to meet requirements as a suitable type of small plant to use for these experiments: once mites have been placed on the plants no chemicals can be used to combat this disease, which can become a serious problem within a few weeks. Of various *Prunus* species which were considered as alternatives, sloe (*Prunus spinosa*) is of irregular growth and has small and variable leaves; myrobalan plum¹) is more suitable, but grows very rapidly, resulting in large plants by the end of the season. *Prunus insititia*²) has proved the most successful for this purpose; seedlings have been grown from yellow bullace, found growing wild in East Anglia, and they are sturdy, relatively slow growing, with large leaves of a suitable texture. *M. ulmi* and several *Typhlodromus* species are found naturally occurring on *Prunus* as well as on apple.

Single seedlings are taken as sampling units, instead of the random leaf samples used in field experiments. Of a number of plants initially inoculated with a known number of each mite species, single ones are taken at regular intervals, the leaves stripped off, and all the mites on them counted under a binocular microscope; the stem is then tapped to remove any mites which remain there. The seedlings are fairly constant in size, and care is taken to sample seedlings of similar leaf numbers at one time. In this way the total number of individuals which results from an initial number of mites is obtained, instead of an average number of mites per leaf, and the sampling error due to variability in leaves is avoided. Before each experiment the seedlings are dipped once or twice in TEPP solution to kill all insects and mites. The stem is then ringed with tree-banding grease to prevent mites walking on or off the plants. There is always some re-contamination by *Tetranychus telarius* L., but not usually until at least six weeks after the start of the experiment, and then it is generally well controlled on any seedlings bearing *Typhlodromus* mites. Known numbers of mites are transferred on to the plants with a brush, and, up to the present, reproducing females of both *M. ulmi* and *Typhlodromus* from field populations have been used. Adults of *M. ulmi* have been taken intentionally, although in the field, for several weeks in May, immature stages only of this species are present and subject to attack by adult

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¹) *Prunus cerasifera* Ehrl.

²) Bullace.

T. tiliae, which come out of hibernation and actively feed and reproduce from April onwards. Adult females and eggs are the stages of *M. ulmi* least attacked by *T. tiliae*, and therefore the phytophagous mite is given the maximum opportunity to become established.

Results

In all figures except Fig. 3 a square root vertical scale has been employed and, except in Fig. 2, totals of active mite stages are used, the numbers of eggs being omitted.

The effect of Typhlodromus tiliae on Metatetranychus ulmi

Fig. 1 illustrates the results obtained in an experiment carried out on *Prunus insititia* in 1957, where three groups of 30 seedlings were taken at the end of May, and on to each plant of each group 5, 25 or 50 *M. ulmi* females respectively were transferred; on to half of the plants of each group five *T. tiliae* females were also added. Seedlings of each group, with and without *T. tiliae* (Fig. 1, A, B, C) were then sampled at weekly intervals until early September. The results were decisive; on the seedlings where no *T. tiliae* were present, *M. ulmi* numbers increased steadily, until within from eight weeks, where there were initially 50 *M. ulmi*, to twelve weeks, where there had been only 5 *M. ulmi* females, the

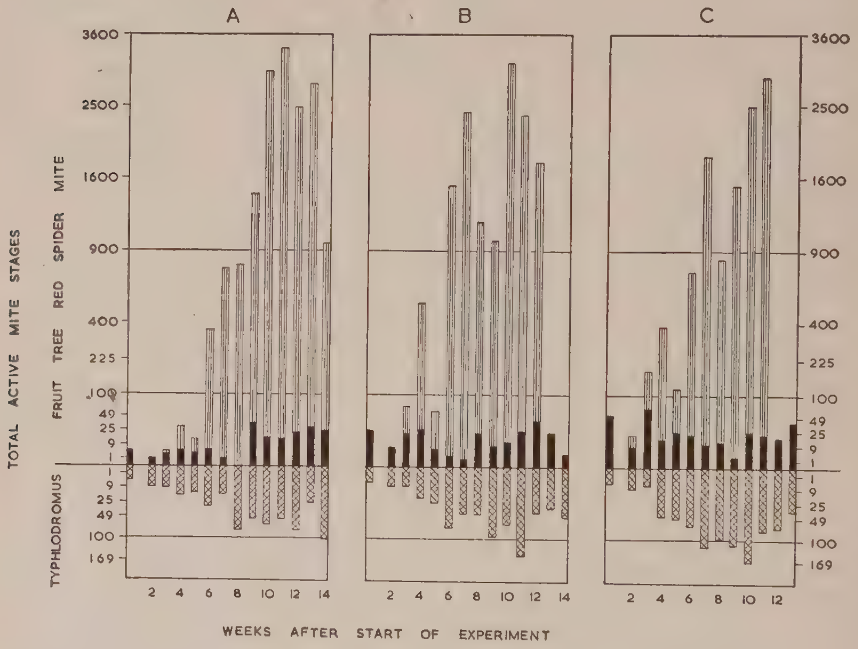

Fig. 1. Effect of *Typhlodromus tiliae* on *Metatetranychus ulmi*, bullace seedlings, 1957. Total numbers of active mite stages per seedling, taken at weekly intervals. Development of populations of *M. ulmi* starting at 5, 25 or 50 mites (A, B and C respectively); black: in the presence of *T. tiliae*; striped: in the absence of *T. tiliae*. Below, cross hatched: numbers of *T. tiliae* developing from 5 mites, with three initial densities of *M. ulmi*.

leaves became bronzed and, later, mite numbers began to fall. The number of leaves per seedling was about twelve at the start of the experiment, and rose through the season to approximately fifty; the maximum number of *M. ulmi* mites that a seedling could support before bronzing and defoliation was in the region of 3,000. Where *T. tiliae* was present, at an initial ratio of 1 : 1, 1 : 5, or 1 : 10 of *M. ulmi*, the plants all remained green and healthy, and *M. ulmi* numbers increased very little; *M. ulmi* never reached a density of one mite per leaf, except for the first three weeks following the initial infestation of 25 and 50 mites per plant, whereas *T. tiliae* itself maintained an average density of from one to three mites per leaf. This latter density is rarely exceeded in this country, either in the insectary or in the orchard.

Experiments carried out on myrobalan and apple seedlings in 1955 and 1956 gave similar results, showing that the host plant does not materially affect the relationship between the two mite species.

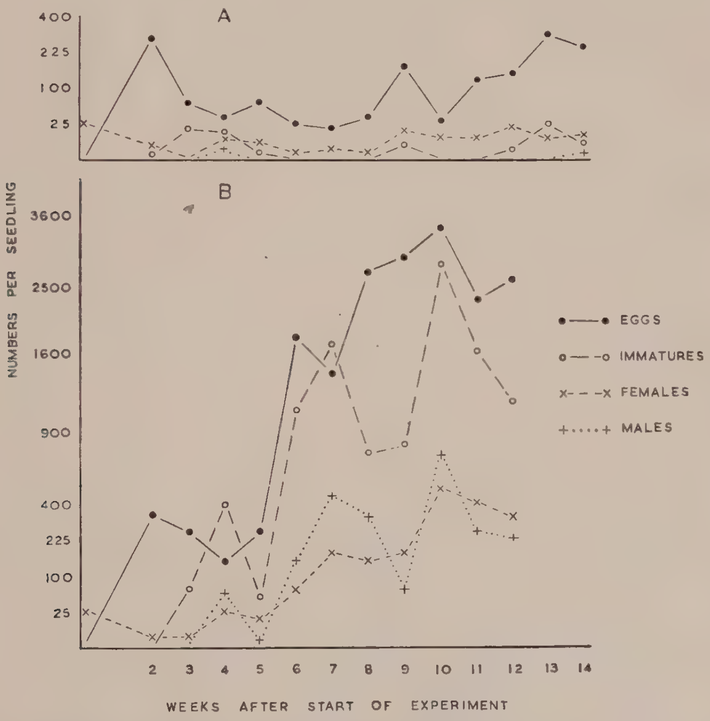

Fig. 2. *Metatetranychus ulmi* populations, broken down into developmental stages. Data from Fig. 1B; development from 25 females: A, in presence of *Typhlodromus tiliae*, starting with 5 females; B, no *T. tiliae*.

In Fig. 2, numbers of eggs, immature stages, females and males developing from 25 *M. ulmi* females in the presence and absence of *T. tiliae* (the complete data from Fig. 1B) are given. On the control plants, a series of peaks for each stage was obtained, showing the development of the generations; the peaks for eggs were highest, for immatures next, while those for adult females and adult males were lowest (Fig. 2B). Where *T. tiliae* was operating (Fig. 2A), the *M. ulmi* population was lower, but once again the eggs were most abundant; by contrast, the immature mites were at a very low level, the adult females exceeding them in number. This difference reflects the very high mortality in the early immature stages where *Typhlodromus* attacks an *M. ulmi* population. The number of eggs laid by 25 *M. ulmi* females in the presence, and absence, of *T. tiliae* during the first two weeks, was very similar, 290 and 340 respectively; it was only after this that the high mortality due to *Typhlodromus* became apparent.

The effect of Metatetranychus ulmi on Typhlodromus tiliae

T. tiliae is partially phytophagous, but needs in addition some more nutritious food. It seemed probable therefore that its development would be dependent on the density of *M. ulmi*, if that were the only food available apart from the plant. Also, as the predacious mite regulates itself to a relatively low number of mites per leaf, it is apparent that the size and growth of the host plant will to some extent affect the development of a population. These factors were considered together in an experiment carried out in 1956 on two host plants; *Prunus spinosa* provided a small plant with relatively slow growth, and it was compared with myrobalan, a larger and more rapidly growing plant. On to plants of each species were placed *T. tiliae* and *M. ulmi* at two ratios 1 : 1 and 1 : 5; in addition three levels of infestation were used for each ratio, giving *T. tiliae* at a starting level of 10, 20 and 50 mites per plant with either the same numbers of *M. ulmi*, or at 50, 100 and 250 *M. ulmi* per plant.

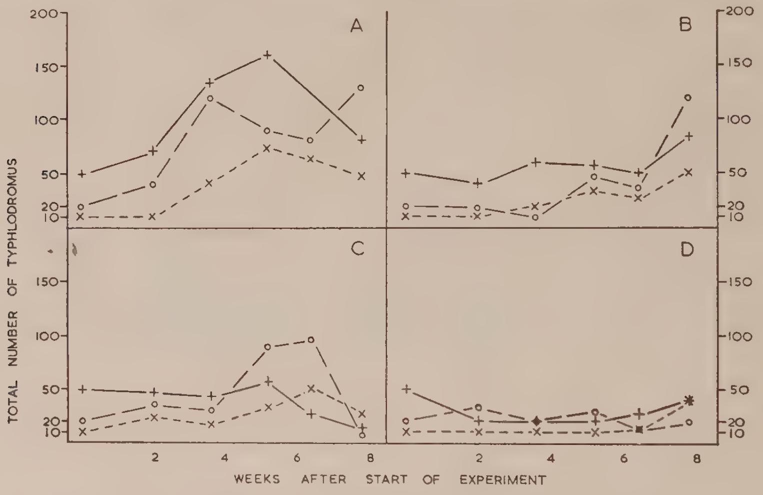

Fig. 3. Development of *Typhlodromus tiliae* populations:
A, B — on myrobalan plants
C, D — on sloe plants
A, C — initial ratio with *M. ulmi* 1 : 5.
B, D — initial ratio with *M. ulmi* 1 : 1.

On the rapidly growing myrobalan, with *M. ulmi* at the 1 : 5 ratio, *T. tiliae* numbers increased relatively rapidly for about five weeks, after which there was a falling off, but the final levels were markedly higher than the initial numbers for all levels of infestation (Fig. 3A). Where the same host plant was used, but *M. ulmi* was initially at the low ratio of 1 : 1, increase of *T. tiliae* was very slow for six weeks, the lower levels showing more increase than the higher; by the final record at eight weeks, the two lower levels reached 5 or 6 times their initial density, while the highest level had not even been doubled (Fig. 3B). Where the small host plant was used (Fig. 3D), there was only a slight increase of *T. tiliae* at the most, and in some cases an actual decrease; the final numbers of *T. tiliae* were low on all the plants, but while there had been some increase of *T. tiliae* at all three levels following the initially higher number of *M. ulmi*, followed by a later drop, where *M. ulmi* was lower there was little change in level throughout the eight weeks. There is shown here a distinct influence of the size of host plant and also an effect of the initial density of *M. ulmi*; it appears that the rate of development of *T. tiliae* is dependent on the amount of food that is present in the form of *M. ulmi* at these low densities, whereas the ultimate level is affected more by the size of the host plant.

The effect of other predacious species on Metatetranychus ulmi

Small experiments were also carried out with other species of *Typhlodromus*, and with one of *Phytoseius*, to compare their influence on *M. ulmi* with that of *T. tiliae*.

Typhlodromus finlandicus (Oudms.) is a species normally found on fruit trees

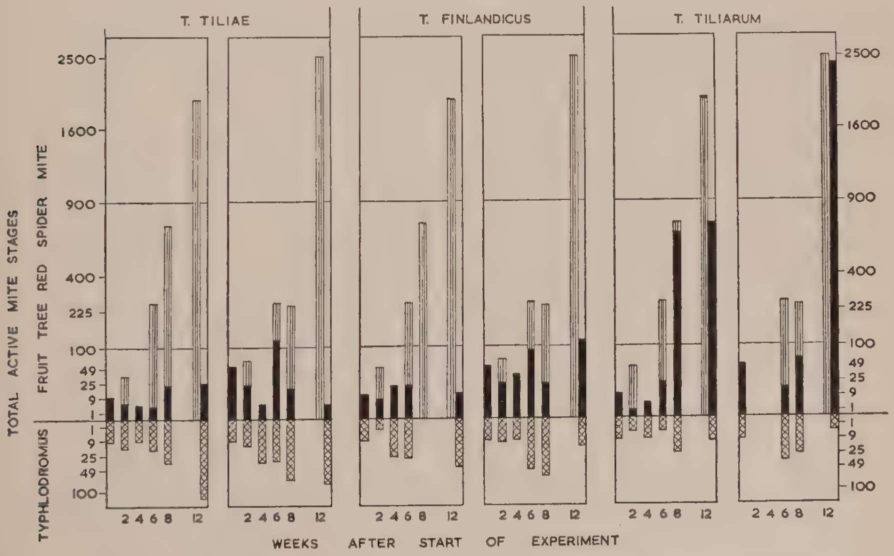

Fig. 4. Effect of other *Typhlodromus* species on *Metatetranychus ulmi*. Apple seedlings, 1956. Explanation as Fig. 1. *Typhlodromus* in all cases started at 10 females per plant, *M. ulmi* at 10 or 50 per plant.

if they have not been sprayed. In an experiment on apple seedlings in 1956 (Fig. 4), which was repeated later on bullace, *T. finlandicus* in the presence of both an equal number of *M. ulmi* and five times as many, increased steadily, and kept *M. ulmi* at a relatively low density. Results were similar to those obtained with *T. tiliae.*

Typhlodromus tiliarum Oudms. occurs less abundantly on both plum and apple trees. It was included in the same experiment in 1956 on apple seedlings, but although it survived and at first fed on *M. ulmi,* this species never increased to any extent, and after an early check *M. ulmi* populations increased to levels not dissimilar to those on seedlings with no *T. tiliarum.*

Typhlodromus umbraticus Chant is normally found on low growing plants, and occurs only occasionally on fruit trees. It thrived on *Prunus* seedlings in the insectary, and in an experiment on bullace in 1955 (Fig. 5) increased at least as well as *T. tiliae,* but appeared to have little effect on the *M. ulmi* population, which also increased steadily, at a rate not far short of that on the control plants.

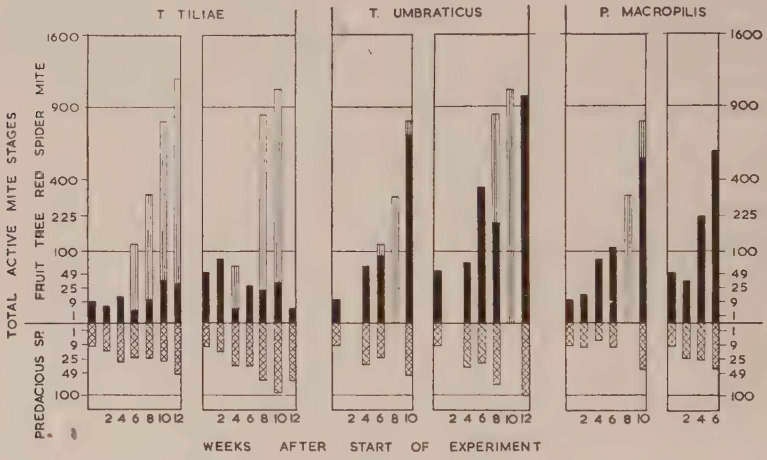

Fig. 5. Effect of other predacious species on *Metatetranychus ulmi.* Myrobalan plants, 1955. Explanation as Fig. 4.

Phytoseius macropilis (Banks) is found on unsprayed fruit trees, but shows a distinct preference for those with hairy leaves. On bullace it developed very slowly at the lower level of *M. ulmi*, and at neither density did it appear to check the phytophagous mite development to any extent (Fig. 5).

Discussion

These experiments have shown that *T. tiliae* and *T. finlandicus* are able to hold *M. ulmi* in check under certain conditions, whereas other species of *Typhlodromus* and *Phytoseius macropilis* are relatively ineffective. *T. tiliae*, the species most common in orchards, can check the increase of an *M. ulmi* population which starts at up to ten times its own level, and hold it through the season at a fairly constant density, rarely exceeding one mite per leaf average, which is lower than the density of *T. tiliae* itself. All numbers referred to are of active mite stages; if eggs were also taken into account the relationship would be somewhat different, but even more striking, as a high proportion of an *M. ulmi* population consists of eggs, which however form a small proportion of a *T. tiliae* population.

COLLYER & KIRBY (1955) postulated from field records of a spraying trial, that if *T. tiliae* and *M. ulmi* were at nearly equal levels, then the predacious mite could control the pest at a constant level, whereas if the ratio became less favourable than 1 : 10, control was not likely to occur. These experiments have shown that this relationship between the two mite species does hold if all other factors are excluded. Moreover, the test of the ability of *T. tiliae* to check *M. ulmi*, which has the greater potential rate of increase, was under conditions made as stringent as possible by starting with adult females of both species. *Typhlodromus* feeds mainly on young red spider mites, so that by taking *M. ulmi* females this mite is given a fair start, the female mites actually laying almost as many eggs in the first generation in the presence of *T. tiliae* as in its absence (Fig. 2). In the field, however, *T. tiliae* adults emerge and feed in April and May, and are fully active during the six weeks hatching period of *M. ulmi* so that the opportunities for control of the pest are far better than in these insectary trials. In field experiments *T. tiliae* does not usually show an increase in response to *M. ulmi* density, as it should if it were acting as an efficient predator at all densities of the pest. As its feeding capacity is limited to a few mites a day, and its actual requirements of *M. ulmi* are lower, no response could be expected if the population of red spider increased beyond a certain relatively low level. By excluding other sources of food in these experiments, it has been shown that *T. tiliae* is density-dependent at low densities of *M. ulmi*.

From the fact that *T. tiliae* can control *M. ulmi* populations under insectary conditions, it can be inferred that it is able to do the same in the orchard. It is difficult to prove that this actually takes place, although much circumstantial evidence makes it appear very probable. If *T. tiliae* is to be allowed to survive in the orchard, by the use of suitable spray programmes, then predacious insects will survive as well, as they are less susceptible to many spray materials than the predacious mites. In a complex of predacious species present under such circumstances, various insects will operate on high densities of *M. ulmi*, and *Typhlodromus* on low densities, thus compensating for adverse effects of other factors.

ZUSAMMENFASSUNG

Man hat aus Freilandversuchen gefolgert, daß gewisse *Typhlodromus*-Arten eine wichtige Rolle bei der Bekämpfung von *Metatetranychus ulmi* (KOCH) spielen können, obwohl die Beurteilung der Ergebnisse nach der Anwendung von Chemikalien und in Gegenwart räuberischer Insekten oft erschwert ist. Um diese Komplikationen auszuschalten, wurden Versuche in einem Insektarium durchgeführt, wo die Wirkung von *Typhlodromus* auf *M. ulmi* in Abwesenheit anderer Beute geschätzt werden kann.

Wenn Ausgangszahlen von 5, 25 oder 50 *M. ulmi*-Weibchen zusammen mit 5 Weibchen von *Typhlodromus tiliae* OUDMS. an kleine Pflanzen gesetzt wurden, blieb die *M. ulmi*-Population während der folgenden drei Monate in jedem Fall niedrig und überstieg die durchschnittliche Dichte von einer Milbe pro Blatt oder eine Summe von 50 Milben pro Pflanze nicht. Wenn die gleichen Anzahlen von *M. ulmi*-Weibchen in Abwesenheit von *T. tiliae* angesetzt wurden, so ergab sich ein jäher Anstieg zu Populationen von über 3000

Milben je Pflanze im Zeitraum bis zu 11 Wochen und bald folgte Bronzefärbung der Blätter.

Die Größe der Wirtspflanzen beeinflußte die Entwicklung der *T. tiliae*-Populationen ebenso wie das Ausgangsverhältnis von *M. ulmi* zu *T. tiliae*. Sowohl größere Wirtspflanzen wie höhere *M. ulmi*-Werte führen zu größeren *T. tiliae*-Zahlen.

Von anderen räuberischen Arten hat *Typhlodromus finlandicus* (OUDMS.) eine ähnliche Wirkung auf *M. ulmi* wie *T. tiliae*. Obwohl *Typhlodromus umbraticus* CHANT sich selbst stetig vermehrte, übte er einen geringen Einfluß auf die *M. ulmi*-Populationen aus; ebenso wenig *Typhlodromus tiliarum* OUDMS., welcher sich jedoch unter den gegebenen Bedingungen nicht gut entwickelte. Auch *Phytoseius macropilis* (BANKS) zeigte nur langsames Wachstum und hatte wenig Wirkung auf die *M. ulmi*-Population.

So wurde nachgewiesen, daß *T. tiliae* unter etwas begrenzten Bedingungen und in Abwesenheit irgendeiner anderen Beute fähig ist, Populationen von *M. ulmi* auf einer relativ niedrigen und gleichbleibenden Dichte zu halten, woraus sich eine Stütze für die Theorie ergibt, daß das auch unter Obstgartenverhältnissen zutrifft.

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INFLUENCE OF SUBLETHAL DOSES OF DDT UPON THE MULTIPLICATION RATE OF *SITOPHILUS GRANARIUS* (COLEOPT. CURCULIONIDAE)

BY

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Sitophilus granarius was exposed to very low doses of DDT mixed with the wheat in which it was reared (0.0, 0.10, 0.125, 0.25 mg DDT per 100 g wheat). The weevils exposed to 0.10 and 0.125 mg produced about 20% more offspring than the unexposed weevils. At 0.25 mg DDT the reproduction was even higher per living female in the first five weeks, but mortality was high and the total number of offspring was much lower than in the other cultures.

The results are compared with previous results, obtained by exposing *Metatetranychus ulmi* (Acari, Tetranychidae) to DDT and it is suggested that the less susceptible an arthropod species is to DDT the greater the possibility that stimulation of reproduction will be found.

In an article by HUECK, KUENEN, DEN BOER & JAEGER-DRAAFSEL (1952) it has been shown that DDT at low concentrations has a stimulating effect on the egg production of the Fruit Tree Red Spider, *Metatetranychus ulmi* Koch (Acari, Tetranychidae).

It was thought that this might not be an isolated instance, but a special case of a general phenomenon and that DDT in sublethal doses might have a stimulating influence on the reproduction of other arthropods.

The case of *M. ulmi* is particular in so far as this mite is "not susceptible to DDT", that is to say, it cannot be controlled with DDT in the field, and in the laboratory too it is not killed by concentrations of DDT which are highly toxic to a great number of insects. Consequently effects other than death can be tested at relatively high doses of DDT.

We therefore now tried to experiment with an insect that is highly susceptible to DDT, but used very low concentrations.

As experimental animal *Sitophilus granarius* L. was chosen. It is easy to rear and can be kept in constant contact with DDT when this is mixed with the grain on which the weevil feeds and in which it oviposits. For some time DDT has been recommended for control of grain weevils in bulk storage but toxicity-hazards to man have stood in the way of widespread use of this method.

TECHNIQUE

S. granarius was mass-cultured on wheat (*Triticum vulgare* L.) in a constant temperature room at 25° C and a relative humidity of 65—70% (= vapour pressure deficit ca 7 mm).

Wheat was sterilized by heat some weeks before the time of the experiment, and subsequently allowed to take up moisture in equilibrium with the rearing room atmosphere.

For an experiment thirty ♂♂ and thirty ♀♀ weevils, 12—15 days old, were put into a glass jar (1.3 l) with 250 g wheat, i.e. about one female per 200 grains of wheat, a concentration at which interference between ovipositing females is slight or negligible (RICHARDS, 1947).

The wheat was either mixed with talcum (35 mg per 100 g wheat) or with the same weight talcum + DDT, containing different concentrations of DDT. For commercial purposes, to control *S. granarius* in bulk wheat, about 10 mg DDT per 100 g wheat is recommended. After a number of failures because the DDT gave too high a mortality we finally adopted the following concentrations:

C_0 = 0.0 mg DDT per 100 g wheat
C_1 = 0.10 ,, ,, ,, ,, ,, ,,
C_2 = 0.125 ,, ,, ,, ,, ,, ,,
C_3 = 0.25 ,, ,, ,, ,, ,, ,,

The weevils were left in the wheat for one week and then transferred to another jar again with 250 g wheat and the same concentration of DDT. At every transfer the number of live weevils was counted. In this way we got a mortality curve of the weevils with continuous exposure to DDT.

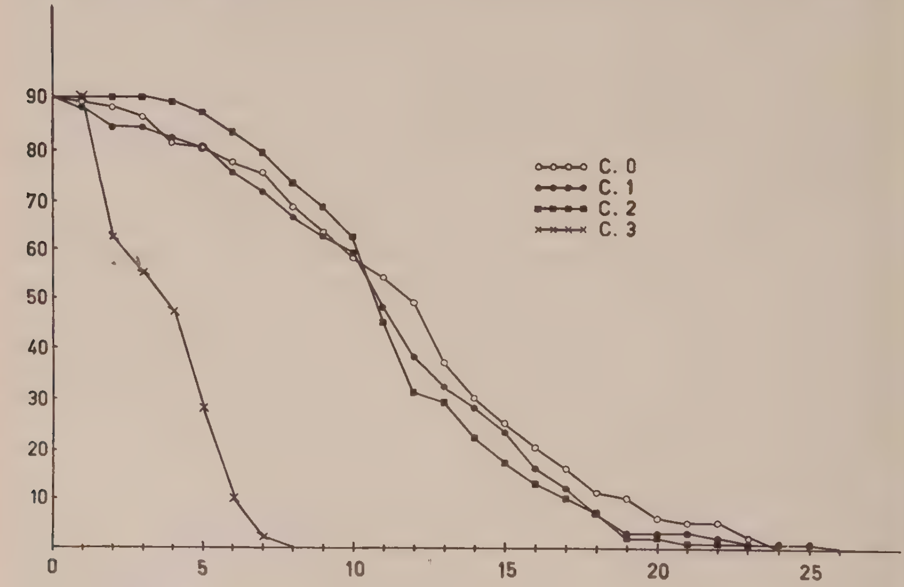

Fig. 1. Abscissa: weeks from beginning of experiment. Ordinate: total number of female weevils present.

Direct counting of the eggs in the wheat is not practical. We therefore waited for the adults to emerge from the wheat and counted these. Each jar, in which the weevils had oviposited for one week, was kept in the same room, and from the fifth week onwards the number of hatched weevils was counted each week. One week is a sufficiently short interval as no oviposition takes place by the newly emerged weevils within this short time. All emerging weevils were therefore F_1 descendents of the original thirty weevils and no F_2 specimens appeared.

The number of weevils that emerge bears some relation to the number of eggs deposited by the weevils. As the whole life cycle from egg to adult takes place within the wheat kernel, the DDT mixed with the grain will not have an appreciable effect on the egg-, larval-, or pupal mortality. As all other factors were equal, as far as possible, the number of adults is a fair indication of the number of eggs produced. The whole experiment was run in triplicate.

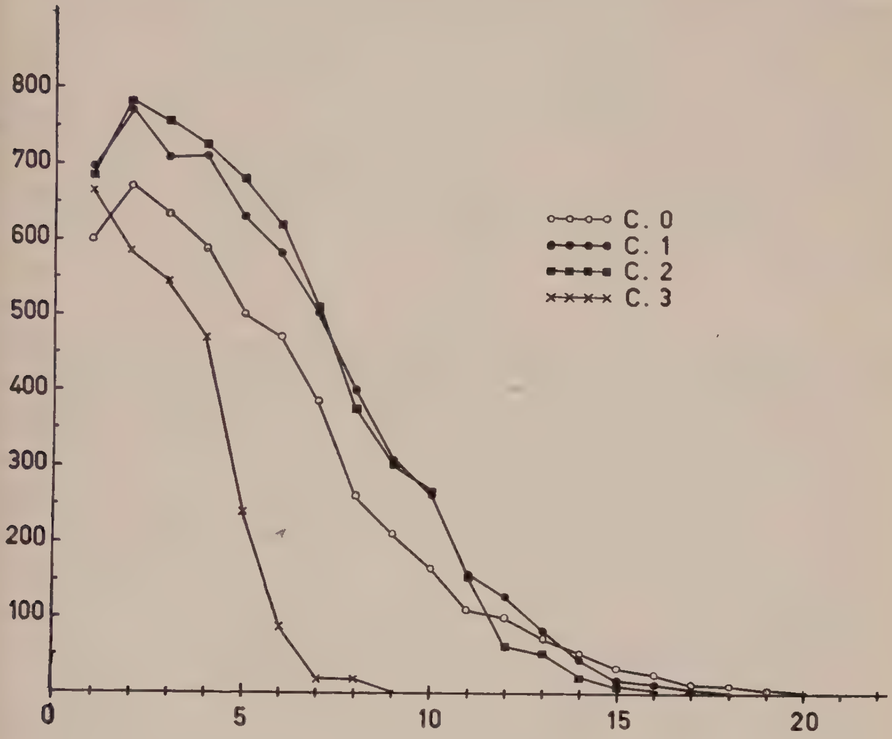

Fig. 2. Abscissa: weeks from first week when adults of the F1 generation were found. Ordinate: total number of F1 adults emerging per week from three jars.

RESULTS

Fig. 1 gives mortality curves of the adult weevils. There was little difference between concentration C_0, C_1 and C_2, though the mortality began about three weeks later in the C_2 jars. At the highest concentration used, C_3, there was considerable mortality and the last weevil alive was found in the seventh week.

Fig. 2 shows the totals of the adults which emerged from the wheat in which oviposition had taken place. The first week in this diagram is the fifth week after oviposition and the first in which an egg could have developed into an adult.

At concentrations C_0, C_1 and C_2 the maximum production was reached in the second week. This maximum is a result of a maximum in egg production 5—6 weeks before.

The number of adults emerging per week was considerably larger in C_1 and C_2 than in C_0. This continued to be so up to the 11th week for C_2 and the 13th week for C_1. The numbers produced after these times, however, were so small that they had little influence on the totals. These are shown in Table I.

TABLE I

Total number of adults emerged

	Number	% increase or decrease
C_0	4962	100
C_1	6062	122
C_2	5956	120
C_3	2638	53

Presumably the numbers of eggs produced by the adults were only slightly higher, as there was very little pre-adult mortality, and such mortality as occurred was of the same relative value in each of the jars.

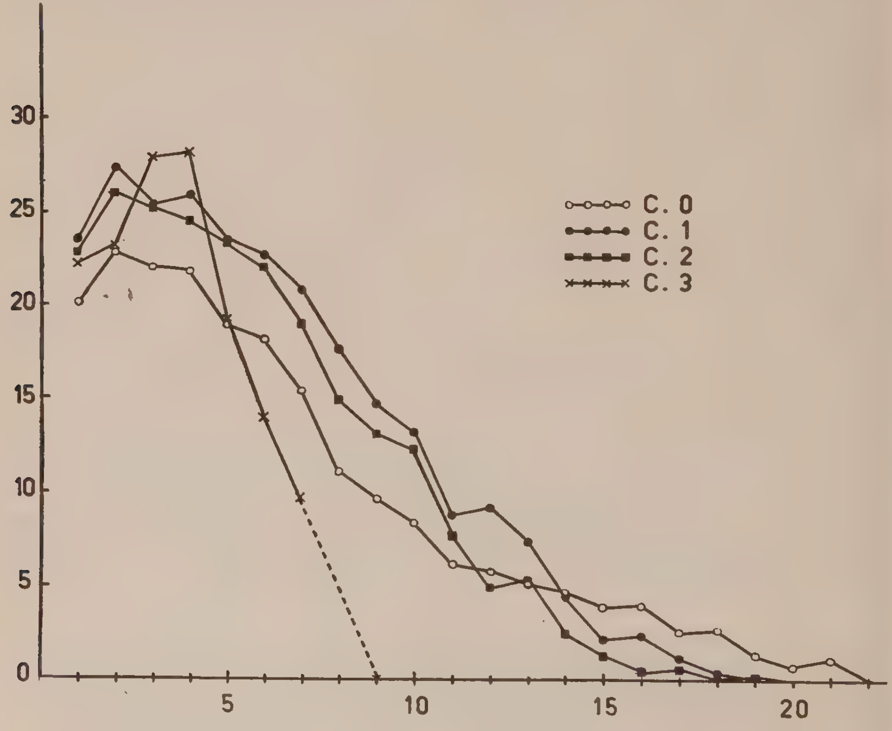

Fig. 3. Abscissa: weeks (as fig. 2). Ordinate: number of F_2 per P_1 female weevil.

The total reproduction under the influence of these low concentrations of DDT (C_1, C_2 but not C_3) therefore was about 20% higher than in the control.

Finally we had to calculate egg production per female, as the differences in mortality rate of the adults cannot be neglected. The number of eggs produced during one week was divided by the mean of the number of living females present at the beginning and at the end of that week, assuming a rectilinear mortality curve during the week (fig. 3).

The curves for mean reproduction for C_0, C_1 and C_2 do not differ substantially from the curves for total egg production (fig. 2). But the curve for C_3 is entirely different. In the first five weeks the low total reproduction at this concentration was entirely due to the high mortality, but the egg production per female reached the highest point of all here. Even the rapid fall in the fifth week brought the production only to the level of the control. Three weeks later a last solitary weevil was present in one of the three parallel jars. No mean egg production can be reasonably calculated for this period, because of these low numbers.

DISCUSSION

The practical implications of these results have first to be considered. Although it is possible that, because of insufficient mixing, sublethal doses may be present locally in bulk wheat when it is treated with DDT, it is very improbable that this would ever have a very disadvantageous result. The mortality will always be so great that the 20% stimulation will be negligible. But if lower doses are used for safety reasons, then stimulation might counterbalance at least part of the mortality.

As LOOSJES (1957) has shown, the parasite *Chaetospila elegans* (Westw.) is quickly killed by DDT, and this action may have considerable influence on the result of the DDT application if the parasite is present. The additive result of such effect may have to be considered as it may come near the level of practical importance.

Of course DDT is not used extensively in weevil control in wheat. But there is no reason to suppose that this reaction of *S. granarius* to DDT is specific, especially as a similar effect was shown to result from application of DDT to a red spider mite. It is worthwhile searching for further evidence of increased egg production where the possibility exists of insects coming into contact with sublethal doses of DDT.

Secondly the physiological aspect of the problem needs consideration. Obviously there are innumerable toxicants with widely different physiological mechanisms. We therefore cannot possibly expect a general rule to exist for all toxicants. But a parallel with many other toxicants and DDT forces itself upon us when we see that low doses have a stimulating effect (on reproduction), while high doses kill the organism.

S. granarius is stimulated to higher reproduction by permanent exposure to 0.10—0.125 mg DDT per 100 g wheat. Mortality is hardly affected. With twice the highest concentration the reproduction is even higher, as long as the females live, but mortality soon intervenes, and reproduction falls to zero. Those individuals which are least susceptible to DDT demonstrate that the larger DDT doses also have a greater stimulating effect.

The Fruit Tree Red Spider, which is not easily killed by DDT shows this higher reproductive rate more easily at much larger doses of DDT.

It is to be expected that the less susceptible an arthropod is to DDT, the more chance there is that the stimulation by DDT will manifest itself.

ZUSAMMENFASSUNG

Sitophilus granarius wurde sehr geringen Dosen von DDT ausgesetzt, die Weizen beigemischt waren, in dem er gezüchtet wurde (0,0, 0,10, 0,125, 0,25 mg DDT auf g Weizen). Käfer, die 0,10 und 0,125 mg erhielten, produzierten über 20% Nachkommen mehr als die Kontrollkäfer. Sogar bei 0,25 mg DDT war die Vermehrung pro lebendem Weibchen in den ersten fünf Wochen höher, aber die Mortalität groß und die Gesamtzahl der Nachkommen viel geringer als in den anderen Zuchten.

Die Resultate werden mit früheren Ergebnissen verglichen, die bei der Behandlung von *Metatetranychus ulmi* (Acari, Tetranychidae) mit DDT erhalten worden waren und es wird angenommen, daß die Möglichkeit, stimulierende Wirkungen zu finden, umso größer ist, je weniger empfindlich eine Arthropoden-Art gegenüber DDT ist.

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THE ALARM REACTION OF MOSQUITO LARVAE

BY

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When *Aedes* larvae are alarmed they swim actively to the bottom; they recover by floating passively upwards. *Culex* and *Anopheles* larvae sink passively when alarmed; they swim actively to the surface on recovery. Repeated stimulation produces "stimulus satiation", but a different type of stimulus can then give a complete response. Geotaxis and phototaxis are concerned in the alarm reaction in *Aedes*; phototaxis can overrule geotaxis. The chill coma temperature of these larvae is several degrees cooler than that at which the alarm reaction takes place. Acclimatization can alter the position of both.

All mosquitoes have aquatic larvae, and the majority of these live at the surface of the water. They lie most of the time with their respiratory syphon penetrating the surface film, and their spiracular system open to the atmosphere. If a shadow passes over the water, or if some mechanical disturbance takes place (e.g. stamping the feet on the ground near a pool, or tapping the edge of a dish in the laboratory) the larvae usually disappear from the surface. This disappearance has been called the "alarm reaction" (BATES 1949). After a period which may differ for different species of mosquitoes, the larvae again return to the surface. The *result* of the alarm reaction is similar for different species, but the *mechanisms* producing this result may vary considerably. The mechanism of recovery is also different in different species. The nature of the stimulus, its frequency, and the conditions of illumination and temperature of the environment all affect the alarm reaction. This paper describes experiments using larvae of the following species; all have been bred in the laboratory for many years:

Aedes (*Stegomyia*) *aegypti* Linnaeus.
Culex (*Culex*) *molestus* Forskål.
Anopheles (*Anopheles*) *maculipennis* Meigen var *atroparvus* Theil.

THE MECHANISM OF THE ALARM REACTION

I. *Aedes aegypti.* Larvae of this species are normally very active, and swim around in a culture dish or in a natural pool far more than do most mosquitoes (JACKSON 1953). If the edge of a dish is tapped once, or if a hand is passed over the surface of the water, the larvae all swim down to the bottom of the dish. If the dish is 20 cm or less deep, almost all the larvae will reach the bottom, and will continue to swim actively near the bottom for some minutes. If no further stimulus is received the larvae then stop swimming and float slowly upwards to

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¹) This work was started in the Entomology Department of the London School of Hygiene & Tropical Medicine, and was financed by the Medical Research Council.

the surface. They may come all the way up without swimming, or they may swim part of the way. In a very large number of experiments using many thousands of larvae, when these had received no other stimulation for several hours, the average time taken to recover from an alarm reaction produced by a single stimulus was approximately four minutes. This species is so active that under favourable temperature conditions we seldom if ever find all individuals motionless at surface, and "recovery" was taken as complete when 75 per cent or more of the individuals were at the surface.

Aedes aegypti larvae are normally lighter than water, and must swim actively to reach the bottom. This means that they must continue to be active during the whole duration of the period of the alarm reaction. Recovery from the alarm reaction may take place when the larva ceases to be active.

II. *Culex molestus.* Larvae of this species are normally much less active than those of *Aedes aegypti.* They hang downwards from the surface film of the water, and may move slowly by means of their brushes. When "alarmed" they usually swim actively for a very short time, and thus withdraw the syphon from the surface. They then lie still, but as they are heavier than the water they sink slowly downwards. They remain without swimming actively for very variable periods, seldom less than one and seldom more than five minutes. They are not as a rule completely motionless, but during this period they give only short and sporadic wriggles. Most larvae in my experiments sank to the bottom of shallow (3 cm) dishes; in deep water few sank deeper than 10 cm before recovery.

Recovery necessitates active swimming by the larvae. They swim quickly up to the surface of the water and re-attach the respiratory syphon to the surface film.

III. *Anopheles maculipennis.* These larvae are even less active than *Culex.* They lie parallel with the surface of the water, often moving slowly by means of the mouth brushes, but seldom swimming at all strongly. When "alarmed" they let go from the surface film and lie completely still, giving the impression of feigning death. As they are quite inactive they sink rather more rapidly than many *Culex* which are delayed in their downward course by each small wriggle. *Anopheles* larvae seem always to be heavier than water.

Recovery takes place after a variable period. The average recovery period for several experiments was similar to *Aedes,* i.e. approximately four minutes, but a minority of larvae may remain motionless on the bottom of the dish for over fifteen minutes.

THE REACTION TO REPEATED STIMULI

It is well known that although mosquito larvae react strongly to stimuli such as shadows and shocks, if these are frequently repeated "stimulus-satiation" or "releaser-satiation" (THORPE 1956) occurs, and the insects cease to react (e.g. see THOMAS 1950, HARPER 1907). This was observed with the three species used in this investigation.

It has been noted that after one stimulus the larvae in a culture of *Aedes aegypti* recovered from the alarm reaction in about four minutes. If instead of tapping the dish once only, this stimulus was repeated frequently, recovery was equally rapid. Thus when the dish was tapped four times a second, and this tapping was continued all the time, the larvae came back to the surface as rapidly as those in the dish given a single stimulus only. If the repeated stimuli were less frequent, being given once a second only, the same result was obtained. But if the period between the taps was increased further, recovery was not so rapid.

At one extreme if one tap only was given, and the larvae were allowed to recover completely (four minutes or longer) a second tap gave a "complete" alarm reaction. When I applied this stimulus once every five minutes for a total period of six hours, the last stimulus gave as complete an alarm reaction as did the first; the only difference was that recovery seemed more rapid as the experiment proceeded.

When the interval between the stimuli was increased from one to five seconds, little recovery was apparent in four minutes, but by fifteen minutes the culture had the same appearance as after four minutes when stimuli were given at intervals of one second only. With fifteen seconds between stimuli recovery occurred in about twenty-seven minutes, with sixty seconds between stimuli recovery took twice as long (i.e. approximately one hour).

A change in the rhythm of the stimuli gave a strong reaction. Thus if the dish were tapped four times a second for five minutes complete recovery was obtained, and the larvae behaved as if no stimulus were being given. If the rate of tapping was then slowed down to one tap per second (a rate at which, had it continued from the start, recovery would have already been complete) a strong alarm reaction took place. If larvae which had recovered with a rapidly repeated stimulus were given no further stimulus for fifteen seconds, a tap after that interval gave a strong reaction once more, but just stopping tapping did not cause a reaction.

Aedes larvae were placed in a water-filled tube 5 cm in diameter and 120 cm long, which was stood vertically. The larvae aggregated normally at the surface. When tapped once, the alarm reaction was elicited. Some larvae swam right to the bottom, others went part of the way down, stopped swimming, and floated up to the surface. If the tube was tapped once a second or more frequently, the same result was obtained, but tapping once every five seconds made all the larvae swim right down to the bottom and remain in the lowest centimetre of water. The longer the period during which the alarm reaction lasted (and this period could be extended by applying the stimuli at longer intervals, provided the intervening period did not exceed one minute), the deeper the larvae continued to swim. This result is similar to that of MACFIE (1923) except that I did not find any falling off in response after several days of exposure, provided that the larvae were not in a room with a lot of mechanical vibration.

The *Aedes* larvae behaved in a similar manner to a different stimulus. As mentioned above, the alarm reaction took place if the hand were moved over the surface of the water. The finding of HOCKING (1953) was confirmed that a narrow object which subtended an angle less than 15° at the head of the larva, or any object which was moved very rapidly, usually gave no reaction. If the hand were moved backwards and forwards about ten cm above the water once a second, the larvae gave the alarm reaction after the first movement, but recovered quite quickly although the stimuli were repeated. Recovery to this stimulus given once a second was as rapid as to taps on the side of the dish at the same intervals. If the period between these visual stimuli was increased, so also was the length of the recovery period, exactly as in the previous tapping experiments.

The following experiment showed that the larvae ceased to give the alarm reaction due to satiation to a particular stimulus, and not because of fatigue. A container with larvae was tapped once a second for four minutes; the larvae by the end of the period had returned to the surface. This stimulus was continued, but at the same time the hand was passed once over the surface of the water. The larvae immediately gave the alarm reaction. The same result was obtained when after four minutes "satiation" to the visual stimulus the dish was given a single tap; the larvae all disappeared from the surface.

Most experiments on repeated stimulation were made with *Aedes*, but a few with the other species of mosquito larvae showed similar behaviour. After tapping a dish of *Culex* larvae once the majority remained at the bottom more-or-less motionless for between two to five minutes. If the dish were tapped once a second throughout this period, recovery was equally rapid. *Culex* larvae satiated with the tap stimulus gave a strong alarm reaction to a visual stimulus. *Anopheles* larvae also recovered as quickly when stimuli were given repeatedly at one-second intervals as they did after a single isolated stimulus.

SOME REACTIONS OF LARVAE TO LIGHT

Several workers have studied the reactions of mosquito larvae to light. Folger (1946) showed that if a moderately bright light was placed at one end of a tank, the larvae aggregated near it, though when stimulated by shock they moved away from light. Thomson (1940) showed that *Anopheles minimus* was repelled by bright sunlight. There is some contraversy about the result of under-water illumination. Miller (1940) found that if *Culex pipiens* were illuminated from below, the smaller larvae remained below the surface and died of drowning. Jobling (1937) found that *Culex pipiens, Culex fatigans* and *Aedes aegypti* could be reared satisfactorily in complete darkness, which suggests that light reactions may not be necessary for normal surface orientation.

I confirmed that the species used in these experiments tended to aggregate at the darker end of a tank exposed in the laboratory. Different reactions were found in the different species to under-water illumination. If *Aedes* larvae were illuminated from the bottom of the tank, they remainded near the surface of the water. Under these conditions tapping did not give the normal alarm reaction. It produced intense activity, but the larvae appeared to be strongly negatively phototactic and remained near the surface. In steady illumination from any side, so long as no other stimulus was given, the larvae remained at the surface, but as far from the source of light as possible. Tapping or other stimuli caused movement away from the light, and this negative phototaxis seemed to overrule any geotaxis. In complete darkness the larvae remained near the surface; tapping now sent them to the bottom in the normal way, suggesting that geotaxis acted normally when not overruled as above.

Aedes and *Anopheles* reacted quite differently to under-water illumination.

When the jar was tapped they behaved very similarly to the way observed in normal overhead light. The *Culex* may have exhibited more activity for a second or more after the stimulus than would have been observed in normal overhead light, but it then relaxed and sank slowly to the bottom, notwithstanding the increasing light gradient to which it was subjected. The *Anopheles* seemed quite unaffected by the direction of the light.

Contrary to the findings of MILLER (1940) it was found that normal development, pupation and emergence took place in cultures of all species illuminated from below. The only failure took place when a large electric bulb under a *Culex* culture raised the temperature of the water to 31° C, a temperature which is too high for this species.

TEMPERATURE AND THE ALARM REACTION

When *Aedes* larvae, cooled down with ice until they were in chill coma, were slowly warmed, it was noticed that spontaneous movement occurred at a temperature several degrees below that at which the alarm reaction was restored. Thus in one group where the temperature at which they emerged from chill coma was 5.7° C, the alarm reaction was not observed until 11° was reached. Between 5.7° C and 11° C the larvae swam, and reacted at once if touched with a needle, though tapping the dish or passing the hand over the surface had no effect.

It has been shown that mosquito larvae, like many other insects, are easily acclimatized to different temperatures (MELLANBY 1939, 1940). If *Aedes aegypti* larvae are kept for a period of 24 hours at a high or a low temperature, the thermal death point (MELLANBY 1954) and the chill coma temperature are substantially altered. So also is the position of the lowest temperature at which the alarm reaction is given, as is shown in Table I.

TABLE I

RESULT OF ACCLIMATIZATION ON THE CHILL COMA TEMPERATURE, AND ON THE TEMPERATURE AT WHICH THE ALARM REACTION TAKES PLACE

	10 ° C	17 ° C	34 ° C
Chill coma	3.4	5.7	10.8
Alarm reaction	under 10 ° C	11.0° C	14.3° C

Similar acclimatization was found in *Culex*, but the limits were not so accurately determined.

I am unable to explain why the threshold temperature for the alarm reaction is so much higher than the chill coma temperature. It appears that the sensory organs the stimulation of which initiates the reaction may require a higher temperature before they function than do the muscles which perform the alarm reaction. It is interesting to note that acclimatization shifts the position of both the chill coma temperature and the alarm threshold temperature in a parallel manner.

It is difficult to observe the alarm reaction at high temperatures. *Aedes aegypti* survives well in water at 35° C, but it is always extremely active. Tapping the dish containing the larvae causes even more activity, but within a few seconds the larvae appear to recover. At these temperatures after an initial period of intense activity the larvae seldom swim more than a few centimetres below the surface ot the water; they will certainly not descend several metres as they will at 20° C.

THE SPECIFIC GRAVITY OF MOSQUITO LARVAE

The differences in the mechanism of the alarm reaction described here are mainly due to differences in specific gravity of the species concerned. *Aedes aegypti* larvae are normally lighter than water, and so can only descend from the surface of the water by active swimming. *Culex* and *Anopheles* larvac are heavier than water; when they leave go of the surface film they naturally sink to the bottom even if they remain motionless. On the other hand *Aedes* return to the surface when they become motionless, but the other two species must swim actively to regain the surface.

These differences in specific gravity are normally found in the majority of larvae, but they are not absolute. If *Aedes* larvae are cooled rapidly to chill coma temperature, more than 75 per cent remain at the top of the water. The remainder may sink to the bottom, but they usually lie vertically just touching the bottom and obviously very little more dense than the water. All *Aedes* larvae can, however, be made to sink. If larvae are kept below the surface by being placed in a submerged cage, at the beginning those which are not actively swimming lie pressed against the top of the cage. Within a few hours (probably when the oxygen in their tracheal trunks has been partially used, and when the carbon dioxide produced has diffused away) the resting larvae sink to the bottom. *Aedes* larvae which, because they have been kept submerged, are heavier than water are much less active than normal larvae, in fact they behave more like *Culex* or *Anopheles* larvae. In well oxygenated water at 20° C they survive for more than 24 hours, but submerged larvae die in a few hours at 35° C. When these larvae which have become heavier than water are released they swim actively upwards to the surface. A few seconds after their respiratory syphons have pierced the surface, the *Aedes* larvae will once more be found to be lighter than the water.

Culex and *Anopheles* larvae are normally heavier than water. In chill coma they all sink and they lie flat on the bottom of the dish; they appear substantially heavier than the water. On the other hand pupae of all three species are normally lighter than water, and when "alarmed" behave in a manner similar to the larvae of *Aedes*.

DISCUSSION

The alarm reaction is presumably of advantage in promoting the survival of mosquito larvae. By submerging they may escape from attack. The experiments described here show that the three species investigated all give an alarm reaction, but that *Aedes* performs the reaction in quite a different way from that exhibited by *Culex* and *Anopheles*, although the end result is the same in both cases. These three species all recover quite quickly after giving the alarm reaction, but as BATES (1949) has shown in his review of the subject, other species react more slowly, and those which live in rapidly flowing water behave somewhat differently. Nevertheless the pattern of reaction in all mosquitoes is not dissimilar; it shows how they are adapted to their particular environments, and how they exploit their different physical peculiarities.

These experiments show that repeated stimulation soon ceases to be effective, and the larvae return to the surface, though a change in the rhythm of stimulation or in the nature of the stimulus itself produces an immediate reaction once more. This again is presumably related to survival; if "stimulus-satiation" did not occur, the larvae might be always on the move and would be unable to feed or respire normally. On the other hand reaction to changes in the tempo of stimulation or in the nature of the stimulus may normally be associated with some new hazard to the larvae.

Larvae exhibit interesting responses to light and to gravity. The experiments with under-water illumination suggest at first that when alarmed the *Aedes*, which remain at the surface, are behaving in a manner fundamentally different from the *Culex* and *Anopheles*, which still sink to the bottom. This difference appears to be due to the fact that *Aedes* is fully active for the whole period of duration of the alarm response, and when active it is negatively phototactic. The other species are immobile for the duration of the response, and so sink normally under gravity (this is not a geotactic response in the ordinary sense) notwithstanding the fact that they are moving into a more brightly illuminated area.

In working on the effects of low temperature, it is often difficult to distinguish clearly between conditions under which normal spontaneous movement occurs, and those under which movement occurs only due to abnormal stimulation. It appears to me that the temperature at which the alarm reaction is restored may be considered to be that at which the insect is able to behave "normally" and that the position of the lower chill coma temperature may be only of academic interest. The difference between these points, and the way in which their level can be rapidly changed by acclimatization for only a few hours (and it must be remembered that the same larva can be adapted to warm conditions on one day, and to cool, on the next, and that this procedure can be repeated and reversed several times) is something which cannot yet be explained, but which may give clues which will eventually help to elucidate the puzzling questions relating to the reaction of life to changes in temperature.

ZUSAMMENFASSUNG

Bei drei Arten von Stechmücken-Larven wurde die Schreck-Reaktion untersucht. Alle Larven verschwinden bei Reiz von der Wasseroberfläche, aber der Reaktions-Mechanismus unterscheidet sich bei den verschiedenen Arten. *Aedes aegypti* ist leichter als Wasser. Die Larven schwimmen aktiv zum Boden und kehren durch Aufwartstreiben passiv wieder zur Wasseroberfläche zurück. Die Larven von *Culex* und *Anopheles* sind schwerer als Wasser. Sie sinken bei Reiz passiv zum Boden, die Rückkehr zur Wasseroberfläche ist ein aktiver Prozess, welcher Schwimmbewegungen der Larven notwendig macht.

Wiederholter Reiz führt zur „Reizsättigung". Hat eine Larve aufgehört, auf einen Anreiz (z.B. Schwingung) zu reagieren, reagiert sie aber sofort auf einen anderen Reiz (z.B. Licht). Dieses deutet an, dass sich die Sinnes-Rezeptoren angepasst haben, dass aber die den Effekt bewirkenden Muskeln nicht ermüdet sind.

Aedes-Larven sind nach Reiz negativ phototaktisch. Sie sind ausserdem positiv geotaktisch, aber die negative Phototaxis ist die stärkere Reaktion. *Culex*- und *Anopheles*-Larven erschlaffen, wenn sie „gereizt" werden, sie sinken in diesem Zustand passiv, sie reagieren nicht auf Wechsel in der Licht-Intensität.

Die zu Aktivitätslosigkeit führende Temperatur ist um einige Grade kälter als die tiefste Temperatur, bei welcher Schreck-Reaktion noch stattfindet. Anpassung, welche vor 24 Stunden erfolgt, kann sowohl die zu Aktivitätslosigkeit führende Temperatur, als auch die Temperatur ändern, bei welcher noch eine Schreck-Reaktion ausgelöst wird.

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EFFECT OF LARVAL POPULATION DENSITY ON THE ADULT MORPHOLOGY OF TWO SPECIES OF LEPIDOPTERA, *PLUSIA GAMMA* L. AND *PIERIS BRASSICAE* L.

BY

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The morphological effects of population density in locusts in the production of 'phases' is now well established and in recent years somewhat parallel effects have been observed in the larvae of Lepidoptera. Unlike locusts Lepidoptera pass through a quiescent pupal period but nevertheless the adult may be affected. In both *P. gamma* and *P. brassicae* adults from crowded larval cultures were smaller in both size and weight although the effect was counteracted to some extent by approaching pupal diapause in *P. brassicae*. The effect was similar in many cases to that produced by an increase in temperature except that it disturbed the relationships between the lengths of the wing and hind femur with the live weight. The ratio of these lengths (equivalent to E/F ratio in locusts) was significantly increased but was not a satisfactory index of larval population density. No effects were observed in the wing pattern and colouration in either species.

Crowding during developmental stages in insects has been shown to have morphological effects on the resulting adults in a wide variety of different insect orders. Wing structures may be profoundly affected. Thus crowding is associated with the production of winged forms in many different species of aphids (GRASSI 1907 cited by ALLEE 1931, DAVIDSON 1914, WADLY 1923) and macropteran forms of the plant-hopper *Nilaparvata lugens* Stal. (KISIMOTO 1956) and of the cowpea weevil *Callosobruchus quadrimaculatus* Fabr. (UTIDA 1954).

Much of the morphometrical information available is about locusts where crowding has been shown to have an effect on many other features as well as the wings such as the length of the femur, the head-width, height and width of the pronotum (UVAROV 1921, FAURE 1932, DUARTE 1938, RAO 1942, ROONWAL 1949, DIRSH 1953). In many of these studies it has been observed that there were differences in the colouration and KEY (1957) found crowding affected colour in addition to femur-length and head-width in three species of Phasmatodea.

FAURE (1943, 1943a) in South Africa and LONG (1953) in Great Britain have shown that crowding has morphological effects in larval stages of Lepidoptera. In the adults of the Army worm *Laphygma exempta* L., FAURE found no morphological differences that could be attributed to the condition of culture. A study to determine if morphometrical differences exist in adult *Plusia gamma* L. and *Pieris brassicae* L. bred in solitary and crowded conditions was therefore undertaken to complement the earlier work on larvae.

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TECHNIQUE

The measurements recommended by the International Locust Conference (Cairo 1936) for the morphometrical study of phase variation in locusts were the lengths of elytron, hind femur and pronotum, the height and minimum width of the pronotum and the maximum width of the head. Of these structures the hind femur and head only are strictly represented in the Lepidoptera and the latter presents difficulties due to the presence of scales. Accordingly the forewing was taken as being equivalent to the elytron in locusts and dimensions were selected to determine its size together with the length of the hind femur.

Two generations each of *P. gamma* and *P. brassicae* were bred in an unheated insectary. The first generation (Experiment I) being in June/July 1956 the mean minimum temperature being 12.5° C (54.5° F) and the second (Experiment II) in Aug./Sept. with a mean minimum temperature of 8.2° C (46.9° F). The larvae were reared in glass beakers as described by LONG (1953) and for purposes of the experiments were set up so that solitary larvae could be contrasted with crowds of sixty per container. Fifty or more adults of each sex and species were used from the solitary condition of culture and a similar number from the crowded condition. Some use was also made of a small number of surplus individuals in a third generation of *P. brassicae* bred in Aug./Sept. 1955, the pupae of which underwent diapause during the following winter and spring (Experiment D).

Freshly formed pupae for both insects were weighed and then kept indoors. Adults were killed by ether vapour within twelve hours of emergence and then weighed. The wings and hind legs were removed. The former were placed between two microscope slides before taking the following measurements.

1. *Body length.* The length was taken from the anterior margin of the head to the posterior margin of the abdomen, by means of dividers and measured to 0.25 mm..
2. *Femur length.* The hind leg was placed on a scaled microscopic slide graduated to 0.25 mm and the length of the femur measured under a binocular microscope.
3. *Wing measurements.* The microscope slides containing the wings were placed on millimeter graph paper and the following wing measurements made by direct-observations.

I) *Costal margin.* The length of the costal margin measured from the humeral plate at the base of the costa to the most distant point on the apex.

II) *Apical margin.* The distance from the apex to the anal angle.

III) *Maximum wing width.* Taken as the maximum width along a line at right angles to the line of the costal measurement.

RESULTS

Effect on size

P. gamma. Throughout the experiments with *P. gamma* the male was found to be generally larger than the female in the measurements taken (Table I) and this difference in size was also found to be reflected in the weights of the pupae and adults (Table II).

TABLE I

MEASUREMENTS OF FORE-WING AND HIND-FEMUR

Mean values and standard errors in mm.

	Experiment No	♂ Solitary	♂ Crowded	♀ Solitary	♀ Crowded
P. gamma					
Length of Costa	I	18.83 ± 0.17	18.11 ± 0.22	18.55 ± 0.01	17.63 ± 0.17
	II	19.55 ± 0.12	19.11 ± 0.08	19.46 ± 0.09	18.57 ± 0.10
Length of Apical Margin	I	11.02 ± 0.12	10.70 ± 0.16	10.91 ± 0.06	10.43 ± 0.14
	II	11.38 ± 0.04	11.09 ± 0.05	11.39 ± 0.08	10.67 ± 0.09
Maximum width	I	8.24 ± 0.05	8.01 ± 0.08	8.16 ± 0.03	8.02 ± 0.05
	II	8.47 ± 0.69	8.32 ± 0.04	8.53 ± 0.06	8.08 ± 0.03
Length of Hind Femur	I	5.55 ± 0.07	5.12 ± 0.13	5.54 ± 0.05	5.07 ± 0.11
	II	5.73 ± 0.05	5.42 ± 0.05	5.70 ± 0.04	5.26 ± 0.05
Costa/Femur Ratio	I	3.395 ± 0.027	3.561 ± 0.066	3.354 ± 0.027	3.512 ± 0.075
	II	3.415 ± 0.023	3.530 ± 0.035	3.419 ± 0.016	3.539 ± 0.031
P. brassicae					
Length of Costa	I	31.78 ± 0.27	30.80 ± 0.16	32.26 ± 0.24	30.52 ± 0.19
	II	33.15 ± 0.16	31.41 ± 0.14	33.62 ± 0.17	32.20 ± 0.25
	D	31.08 ± 0.20	30.17 ± 0.30	31.19 ± 0.27	30.65 ± 0.18
Length of Apical Margin	I	19.31 ± 0.10	18.42 ± 0.10	19.72 ± 0.15	18.40 ± 0.11
	II	20.91 ± 0.17	19.16 ± 0.15	21.41 ± 0.19	19.93 ± 0.20
	D	18.57 ± 0.22	17.97 ± 0.44	18.77 ± 0.18	18.61 ± 0.19
Maximum Width	I	17.79 ± 0.16	17.11 ± 0.07	18.40 ± 0.14	17.35 ± 0.12
	II	18.86 ± 0.11	17.56 ± 0.10	19.55 ± 0.16	18.20 ± 0.17
	D	17.23 ± 0.18	16.54 ± 0.37	17.53 ± 0.18	17.41 ± 0.13
Length of Hind Femur	I	3.64 ± 0.04	3.39 ± 0.03	3.58 ± 0.04	3.33 ± 0.03
	II	3.92 ± 0.04	3.58 ± 0.02	3.88 ± 0.04	3.61 ± 0.03
	D	3.37 ± 0.05	3.32 ± 0.04	3.36 ± 0.03	3.26 ± 0.03
Length of Body	I	24.19 ± 0.20	22.84 ± 0.21	22.94 ± 0.31	21.50 ± 0.18
	II	24.82 ± 0.22	23.55 ± 0.20	23.51 ± 0.22	22.75 ± 0.28
Costa/Femur Ratio	I	8.761 ± 0.055	9.102 ± 0.078	8.847 ± 0.066	9.233 ± 0.080
	II	8.467 ± 0.077	8.760 ± 0.049	8.669 ± 0.066	8.896 ± 0.061
	D	9.223 ± 0.081	9.088 ± 0.093	9.283 ± 0.072	9.345 ± 0.060

Larval crowding, when compared with the solitary treatment, resulted in the production of a smaller adult for not only were reductions up to five per cent

TABLE II

LIVE WEIGHT AT PUPATION AND SUBSEQUENT EMERGENCE IN MG.

	Experiment No	♂ Solitary	♂ Crowded	♀ Solitary	♀ Crowded
P. gamma					
Pupae	I	311.9	280.7	294.8	270.5
Adults	I	150.7	124.0	143.5	118.7
	II	164.1	141.3	161.3	129.1
P. brassicae					
Pupae	I	433.2	377.5	439.6	366.5
	D*	415.1	425.9	423.6	420.0
Adults	I	204.7	169.1	201.5	161.6
	II	220.5	191.7	215.8	190.5
	D*	166.3	170.2	180.5	177.0

* Generation which diapaused as pupae.

TABLE III

THE PERCENTAGE REDUCTION OF THE DIMENSIONS IN SOLITARY CULTURES PRODUCED BY CROWDING

		P. gamma		*P. brassicae*	
		♂	♀	♂	♀
Length of Costa	I	3.82	4.96	3.08	5.39
	II	2.25	4.57	5.25	4.22
	D	—	—	2.93	1.73*
Length of Apical Margin	I	2.91*	4.40	4.61	6.69
	II	2.55	6.32	8.37	6.91
	D	—	—	3.23*	8.52*
Maximum Width	I	2.79	1.72*	3.84	5.71
	II	1.77	5.28	6.90	6.91
	D	—	—	4.01*	6.35*
Length of Hind Femur	I	7.75	8.48	6.87	6.98
	II	5.41	8.48	8.67	6.96
	D	—	—	1.87*	0.24*
Length of Body	I	—	—	5.58	6.28
	II	—	—	5.12	3.23
Costa/Femur Ratio	I	—4.89	—4.71	—3.89	—4.36
	II	—3.37	—4.71	—3.46	—2.62
	D	—	—	+0.13*	—0.06*

* Not significant at $P = < 0.05$

observed in the dimensions recorded (Table III) but reductions from 14 to 20 per cent were obtained in mean weights of the newly emerged adults. Smaller reductions from 8 to 10 per cent had previously been observed in the mean weights of the pupae (Table IV), and the larger percentage adult reductions were due to relatively greater losses in weight in the pupal stage. The pupal loss in weight was a little over 50 per cent in solitary cultures whilst in crowded cultures it was nearly five per cent greater. The reduction in size was greater in the female and this was more pronounced in the measurements for experiment II. This greater effect on the size of the female however was not observed in the effect on the weight in experiment I. The general effect of crowding on the dimensions was to produce a shorter wing with slight variations in the relative widths (Fig. 1). Fairly consistent results were obtained for the extent of the effect on the lengths of the

TABLE IV

THE PERCENTAGE REDUCTION OF THE WEIGHTS IN SOLITARY CULTURES AND THE EXTRA PERCENTAGE LOSS IN WEIGHT DURING THE PUPAL STAGE PRODUCED BY CROWDING

	Experiment No	*P. gamma*		*P. brassicae*	
		♂	♀	♂	♀
Pupae	I	10.0	8.2	12.9	16.6
Adults	I	17.7	17.3	17.4	19.8
	II	13.9	20.0	13.1	11.7
Extra loss in weight in pupal stage	I	4.1	4.8	2.4	1.7

costal margin and the femur, the females being the less variable. The apical margin and the wing-width however showed a wider range of variation.

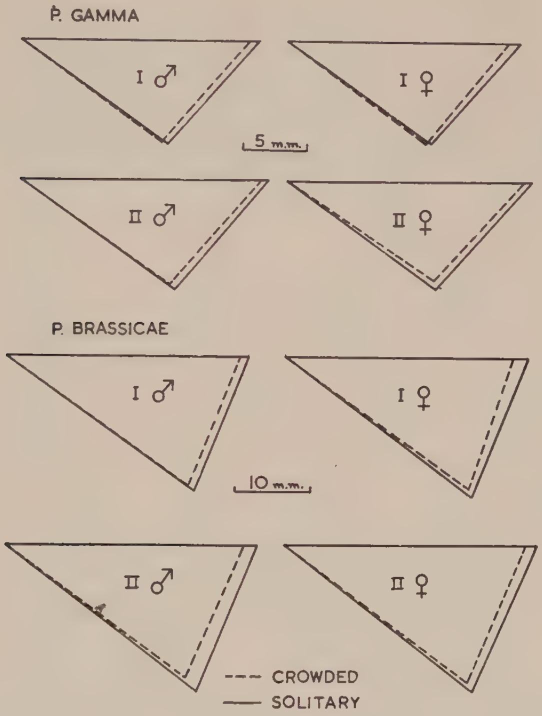

Fig. 1. The effect of larval crowding on wing shape and size in *P. gamma* and *P. brassicae.*

Effect on size

P. brassicae. Although the male of *P. brassicae* was heavier than the female (Table II) and had greater body and femur lengths, the female wing was found to be larger (Table I). Crowding produced a smaller adult with reductions of up to 7 per cent in the dimensions (Table III) from 12 to 20 per cent in the weight (Table IV). There was a slightly greater loss in weight during the pupal stage in crowded cultures but this was not so evident as in *P. gamma.* The reductions in the dimensions were slightly larger and less variable in the female. The wing in the crowded cultures was shorter and relatively narrower in both sexes (Fig. 1). The effect was the most consistent for the lengths of the costal margin and femur and the largest obtained was that for the male femur in experiment II. In general there was a tendency for the effect to be greater in experiment II. In the diapausing generation the males in the crowded culture both as pupae and adults weighed more than their solitary counterparts whilst negligible differences occurred between the females. Nevertheless the dimensions in the adult in both sexes of crowded were less than those in solitary culture although to a smaller extent than that found in the non-diapausing cultures.

Seasonal variations

P. gamma. A comparison of the two experiments showed that the adults in experiment II were larger than those in experiment I in both dimensions (Table V) and weight (Table II). These differences presumably reflect the effect of the different seasons when the experiments were carried out. In the solitary cultures, where the seasonal effect was not confounded with the effect of crowding, the male was affected to a very similar extent in both the wing and the femur whereas the female wing was affected more and the female femur less. The wing was shorter but its shape did not appear to be affected (Fig. 2).

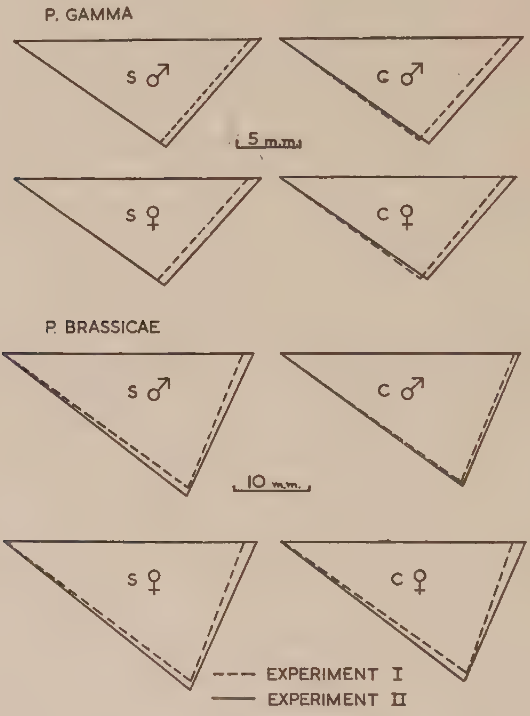

Fig. 2. Variation in wing shape and size with season in *P. gamma* and *P. brassicae*. (For details see text.)

The effect of the season in the crowded cultures appeared to be accentuated in respect of the femur but in general with the exception of the male wing the extent of the effect was less than that obtained by crowding.

Seasonal variations

P. brassicae. As in *P. gamma*, larger adults in both weight and size were obtained in experiment II with *P. brassicae* (Tables II and V). In solitary cultures, apart from the femur which was affected slightly more in the female, no differences in the degree of effect were observed between the sexes.

The wings in experiment I were generally shorter, relatively narrower and differed slightly in shape (Fig. 2). In the female the effect on the wing and also that on the femur was not altered by crowding but in the male the effect was reduced appreciably. The extent of the seasonal effect appeared to be of the same order as that produced by crowding.

TABLE V

THE PERCENTAGE REDUCTION OF THE DIMENSIONS IN EXPERIMENT II ATTRIBUTABLE TO THE WARMER SEASON OCCURRING DURING EXPERIMENT I

	Condition of culture	*P. gamma*		*P. brassicae*	
		♂	♀	♂	♀
Length of	Solitary	3.68	4.68	4.31	4.22
Costa	Crowded	2.62	5.06	1.98	5.50
Length of	Solitary	3.16	4.21	8.29	8.22
Apical Margin	Crowded	2.79	2.25*	4.02	8.32
Maximum	Solitary	2.72	4.34	6.01	6.25
Width	Crowded	3.73	0.74	2.63	4.90
Length of	Solitary	3.14	2.63	7.69	8.38
Hind Femur	Crowded	5.54	3.61	5.61	8.41
Length of	Solitary	—	—	2.60	2.48*
Body	Crowded	—	—	3.11	5.81
Costa/Femur	Solitary	5.86*	1.90	—3.36	2.01*
Ratio	Crowded	—0.88	0.76	—3.76	—3.65

* Not significant at $P = < 0.05$

The relationship of the lengths of the costa and hind femur with the live body weight.

P. gamma. Both the lengths of the costa and hind femur in the solitary cultures were found to have a positive correlation with live weight for both sexes but the regression values were significant in only four out of the eight sets of observations (Table VI). In the solitary cultures the values were larger for experiment II than for experiment I whereas in the crowds with one exception they were smaller. The values for the crowds generally tended to be smaller than their solitary counterparts and only one was significant.

TABLE VI

REGRESSION VALUES (B) FOR THE RELATIONSHIP OF THE LENGTHS OF COSTA AND HIND FEMUR WITH THE LIVE BODY WEIGHT

	Sex	Experiment No.	Costa		Hind-femur	
			Solitaries	Crowds	Solitaries	Crowds
P. gamma						
	♂	I	.02108*	.01405	.00454	.00398
		II	.03249	.00253	.00939*	.00101
	♀	I	.00582	.02967*	.00403	.00691
		II	.02374*	.00812	.00756*	.00945
P. brassicae						
	♂	I	.01454*	.00944	.00005	.00005
		II	.01009	.00353	.00265	.00041
	♀	I	.00389	.01333	.00571*	.00070
		II	.02713*	—.00138	.00411*	.00114

* Significant at $P = < 0.05$

P. brassicae. Similar results were obtained to those for *P. gamma.* In the solitary cultures four out of the eight regressions were significant and the values were generally larger than those obtained for the crowds (Table VI). None of the latter values were significant. The results did not show the seasonal trends observed in *P. gamma* although they did suggest that as in *P. gamma* the value for the costa was smaller in the crowds of experiment II.

The costa/femur ratio

P. gamma. Crowding had a consistent effect on the costa/femur ratio in producing significantly higher values (Tables I and III). The ratios were very similar in both sexes and appeared to be affected by crowding to similar extents.

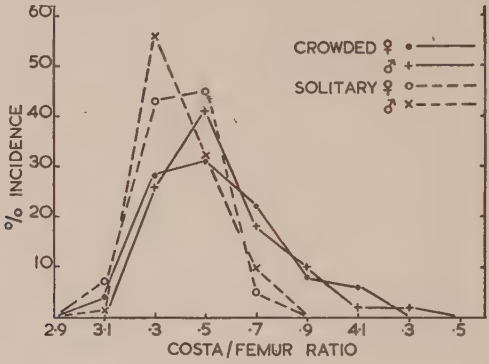

Fig. 3. Distribution curves in *P. gamma* for the incidence of costa/femur ratios in the two different treatments in experiment II.

However the costa/femur ratio did not prove a satisfactory index of the density of larval culture due to the extensive overlap of the values as shown in fig. 3. Crowding did not displace the distribution curves but merely extended their range.

P. brassicae. Significantly higher values for the costa/femur ratio were obtained

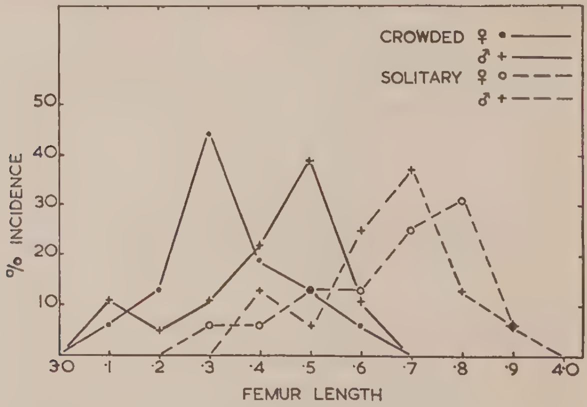

Fig. 4. Distribution curves in *P. brassicae* for the incidence of femur lengths in the two different treatments in cxperiment I.

for non-diapausing crowded cultures (Table I). The value for this ratio was greater for the female in both solitary and crowded cultures and the effect of crowding was less in experiment II (Table III). As in *P. gamma* the costa/femur ratio did not prove a good index of the effect of larval crowding due to the overlap of values. Crowding had its maximum effect on the length of the femur and this gave the best separation of the distribution curves as shown in fig 4.

Effect on the wing pattern and colour

In the course of these experiments and others numerous adults of both species have been examined but no differences in wing pattern and colour attributable to the conditions of culture were detected.

DISCUSSION

The larval lives of *P. gamma* and *P. brassicae* differ in many ways and the factors which lead to the formation and maintenance of aggregations of young larvae in the field, where conditions of crowding may prevail, are quite dissimilar in the two species (LONG 1955). Whereas the isolated individual may be more typical of the normal tendency in *P. gamma* a closely packed aggregation most closely represents the normal experience in *P. brassicae.* It may therefore appear somewhat surprising that crowded conditions have in the past been found to produce certain similar effects in both species of larvae (LONG 1953) and have now been found largely in affect the pupae and adults in a like manner (ZAHER & LONG 1958, LONG & ZAHER 1959).

It is certainly evident that crowding produced a smaller adult in both species. Since it had a larger effect on the females, which in the solitary cultures at emergence weighed less than the males, it tended to accentuate the differences between the sexes. The results of IWAO (1956) for the True Army Worm (*Leucania unipuncta* Haworth) showed that, although the crowded larvae were reared at a much lower density, there was a tendency for crowding to produce a smaller adult and to affect the female more than the male. Following the theory put forward by UVAROV (1921), the phases of locusts have become generally recognised as being attributable to the population density such that, whereas ph. *solitaria* may appear in conditions of relative isolation, crowding leads to the production of ph. *gregaria.* In *Locusta pardalina* ph. *gregaria* is larger than ph. *solitaria* but in *L. migratoria migratorioides* (R & F) it is smaller and reductions in size are bigger in the female (FAURE 1932; DUARTE 1938). Likewise DIRSH (1953) showed the female ph. *gregaria* of *Schistocerca gregaria* (Forskal) to be smaller than ph. *solitaria* in 17 out of 20 different measurements whilst the male was smaller in only 8 measurements. These observations would suggest that this differential effect of crowding on size in relation to sex may involve a principle that is widely applicable. EIGENBRODT (1925 cited by ALLEE 1931) and MILLER & THOMAS (1958) observed that crowding in *Drosophila* caused a reduction in adult size affecting the sexes unequally but this might possibly be attributed to an effect on the quantity and quality of food available. However it would not appear that food limitation was responsible for the effects observed in

Lepidoptera for it has been shown (LONG 1953, 1955; IWAO 1956a) that not only did crowded cultures spend more time feeding but consumed more food.

It has long been recognised that insects bred under warm conditions are frequently smaller than those kept in a cooler environment. HERSH & WARD (1932) found that with an increase of temperature there was a reduction in size of the long-winged type of *Drosophila melanogaster.* GOLIGHTLY & LLOYD (1939) noted a decrease in size in four species of Diptera during the warmer months. This effect of temperature was no doubt largely responsible for the smaller adults obtained in experiment I where the mean minimum temperature was 12.5° C (54.5° F) in contrast to 8.2° C (46.9° F) in experiment II. Temperature also affects developmental stages other than the adult for DOBSON (1955) found the volume of the eggs of the flea beetle *Psylliodes chrysocephala* L. decreased with increasing temperature. If distinction is made between the stage of development and the extent of growth, as reflected by size, these observations suggest that higher temperatures accelerate the rate of development more than the rate of growth.

Crowding has been shown to accelerate the rate of larval development (LONG 1953, HIRATA 1956, IWAO 1956) but slightly smaller pupae were obtained presumably due to the rate of growth not being affected to the same extent. The larger differences in weight subsequently observed between the newly emerged adults from the two conditions of culture reflect the relatively greater weight losses that occurred in the pupal stage in crowded cultures.

Crowding led to a significant increase in the costa/femur ratio in both species but this ratio did not prove a satisfactory index of the conditions of culture due to the wide overlap in values. Likewise in the Acrididae crowding has led to the increases in the elytra/femur ratio associated with the characteristics of the phase *gregaria* of locusts but this too has not proved the best index (FAURE 1932, ROONWAL 1949, DIRSH 1953). In the experiments with Lepidoptera crowding caused a relative decrease in the length of the femur which was nearly twice that of the costal margin thereby producing the differences observed in the costa/femur ratio. A satisfactory ratio however would be more likely to be obtained by contrasting a structure that was decreased with one that was increased in size by crowding such as that shown by KEY (1957) for the femur length and headwidth of the males of the stick insect *Podacanthus wilkinsoni* Macl.

In the experiments with *P. gamma* and *P. brassicae* crowding led to significant reductions in the lengths of the costal margins in both males and females. Although in the solitary cultures there was a general correlation between these dimensions and weight only eight out of the sixteen regressions were significant. In the crowded cultures, however, this relationship with weight was disturbed, only one regression value being significant and on eleven out of sixteen occasions the values were smaller than their solitary counterparts. Crowding appears to disturb normal growth relationships for it was observed earlier for *P. gamma* (LONG 1953) that it disturbed the positive correlation between pupal weight and larval duration. In this respect it does not resemble the effect of increased temperature.

The effect of crowding on the diapausing generation of *P. brassicae* was of singular interest. The differences between the adults from solitary and crowded cultures were for the most part small and with one exception not significant. If however the measurements are compared with those of the non-diapausing generations the solitary males and females were smaller than those in the solitary non-diapausing generations whilst crowded individuals were approximately the same in both generations. This similarity in crowded cultures was associated with the fact that whereas the solitary diapausing pupa was 8 per cent heavier than its non-diapausing counterpart the crowded diapausing pupa was 20 per cent heavier. This suggests that approaching diapause produces changes in the larvae which has a greater effect than the population density. However it does not preclude an interactance between the effects of crowding and diapause for Iwao (1956b) found that the population density was responsible for the induction of diapause in the Rice Green Caterpillar, *Naranga aenescens* Moore.

As the effects of population density are studied in an increasingly wide variety of insect forms it is becoming apparent that there may be a common range of factors which can be affected. In many respects the responses of *P. gamma* and *P. brassicae* to crowding are similar but it bas been shown here and elsewhere (Long 1953, 1955) that differences do occur. Furthermore, it was shown that even within the single species of *P. gamma* there were some larvae which did not show morphological changes while others did so. Hence the differences in response between *P. gamma* and *P. brassicae* serve to illustrate that the precise direction and effect of crowding, if any, on any one factor will depend on a genetically controlled ability to respond in the individual animal. From this it would follow that the ecological results of the total pattern of response would at least be peculiar to the particular species.

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ZUSAMMENFASSUNG

Die Einwirkung der larvalen Populationsdichte auf die Morphologie der jungen Falter wurde bei *Plusia gamma* und *Pieris brassicae* durch Vergleich frischgeschlüpfter Falter aus Einzel- und aus Massenzuchten bestimmt.

Bei *P. gamma* wogen Puppen aus Massenkulturen etwas weniger und verloren während des Puppenstadiums mehr an Gewicht. Die entstehenden Falter waren gewichtsmäßig deutlich leichter und hatten etwas kürzere Flügel. Größe und Gewicht der Männchen waren größer als die der Weibchen, und da die Massenzucht die meßbaren Größen bei den Weibchen stärker beeinflußt, verstärkte sie den Unterschied zwischen den Geschlechtern. Jahreszeitliche Unterschiede wurden beobachtet, wobei Gewicht und Größen in der warmen Jahreszeit geringer waren, doch blieb der jahreszeitliche Einfluß geringer als der durch Übervölkerung hervorgerufene.

Bei *P. brassicae* hatte die Massenzucht eine ähnliche Wirkung auf die Puppen- und Erwachsenengewichte und auch auf die Maße, außer daß der Flügel kürzer und schmaler war. Die Unterschiede zwischen den Geschlechtern wurden durch die Massenaufzucht nicht verschärft. Lebendgewicht und Femurlänge waren beim Männchen größer, währen das Weibchen größere Flügel hatte. Bei Herannahen der Diapause wurde die Wirkung der Massenkultur auf Puppen- und Imaginalgewicht kompensiert, aber die Wirkungen auf die Erwachsenenmaße

blieben noch erhalten, wenn auch in geringerem Ausmaß als sie bei nichtdiapausierenden Generationen zu beobachten waren. In der wärmeren Jahreszeit wurden Variationen erzielt, die in etwa den durch Übervölkerung bewirkten ähnlich waren, außer daß die Flügel abweichende Form aufwiesen und daß Übervölkerung bei den Männchen die Wirkung stark reduzierte.

In Einzelkulturen beider Arten wurden positive, aber nicht stark signifikante Korrelationen in der Beziehung von Costa- und Hinterschenkellänge mit dem Lebendgewicht gefunden. Diese Beziehungen wurden durch Massenkultur gestört.

In beiden Arten wurde das Costa-Schenkel-Verhältnis durch Massenkultur signifikant vergrößert (cf. E/F-Rate bei Heuschrecken), ergab aber keinen befriedigenden Index für die larvale Populationsdichte.

In der Flügelzeichnung und Färbung beider Arten wurden durch larvale Massenkultur keine Wirkungen beobachtet.

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HOST FINDING OF *CHOETOSPILA ELEGANS* WESTW. (HYM. CHALCID.) A PARASITE OF *SITOPHILUS GRANARIUS* L. (COLEOPT. CURCUL.)

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The parasite *Choetospila elegans* is an ectoparasite on Coleoptera larvae and was studied in relation to *Sitophilus granarius*. Only 3rd and 4th stage larvae are parasitized, as a rule, and it is possible that late 2nd stage larvae are occasionally attacked.

Larvae within grain are found by detection of mechanical stimuli set up by the larvae's activity which can be imitated by other animals moving within a hollow grain. This agrees with the fact that the parasites choose grains containing the largest of the active stages of the weevil.

The Chalcidid *Choetospila elegans* was first described by WESTWOOD (1874). After a period of increasing nomenclatorial complications, GAHAN (1946) cleared up the situation, reviewing a number of Chalcidid genera, and placed the genus *Choetospila* in the subfamily Cerocephalinae of the Pteromalidae, a subfamily which parasitizes Coleoptera.

In 1953 this parasite appeared accidently in a culture of the grain weevil *Sitophilus granarius* L. in our laboratory. We have no clue as to where this unbidden guest came from, but started control measures as soon as it developed to any extent. In a few months the infection was reduced by making the containers in which *Sitophilus* was reared parasite-proof, and *Choetospila* had to be kept in stock by deliberate infections. Later this was stopped and the parasite became extinct in our laboratory.

During this period we studied a few aspects of the biology of this parasite which will be partly reported here. The questions presented by the relation between a parasite and its host are innumerable. As our time was limited, we restricted ourselves to the following questions:

1. Which stages of the host can be parasitized effectively ?
2. How are these stages recognized by the parasite ?

We think that answers to these questions have wider significance than the mere factual data relating to these two species.

THE HOST, SITOPHILUS GRANARIUS L.

S. granarius was reared in a room at 25° C. and 70% R.H. Wheat was used as oviposition medium in which development up to the adult stage took place. It also served as food for the adults.

Data on the duration of each of the developmental stages can be found in EASTHAM & SEGROVE (1947) and are tabulated below (Table I). Many details of the biology of this weevil can further be found in RICHARDS (1947).

TABLE I

(EASTHAM & SEGROVE, 1947)

Average duration in days of development of *S. granarius* stages at 25° C, R.H. 70%

egg	4.5
larva I	6.0
larva II	3.5
larva III	3.5
larva IV	11.0
pupa	8.0
total	36.5

We measured only the total duration of development to obtain the data of Table II.

TABLE II

Time of appearance of adults of *S. granarius* after oviposition

days after oviposition	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	
number of weevils	3	9	41	53	34	30	34	21	9	12	3	1	1	1	—	total 252

Emergence was spread over 14 days with a mean duration of development of 38 days. This agrees reasonably well with the data of EASTHAM & SEGROVE. We did not determine the variability in duration of each of the stages. When the period of total development was longer than 36.5 days we assumed a proportional lengthening of each stage.

THE PARASITE, CHOETOSPILA ELEGANS WESTW.

This parasite gives a somewhat antlike impression which is strengthened by the fact that it does not fly readily. The females are slightly larger than the males: ♀♀ 0.7—2.1 mm; ♂♂ 0.6—1.9 mm. Large parts of head and thorax, and the entire abdomen are black with a metallic shine. The wings are clear except for a dark bar across them. The forewings have a conspicuous black conical bundle of hairs projecting forward.

Ch. elegans has been reported from many tropical countries and also from warehouses in temperate areas, the hosts being different species of Coleoptera.

BARE (1942) gave the period of development as 18—23 days from egg to adult with *Lasioderma serricorne* L. as host. We have not been able to find any other record of the period of development.

We found the development of egg + larva to take about 10 days at 25° C and 70% R.H. The pupa acquires its pigmentation in about 7 days. The males begin to emerge from the 19th day after oviposition, the females on the 21st. Half the population emerges before the 25th day. Females live 2—3 weeks under the given circumstances; males somewhat less.

Several authors express the opinion that *Choetospila* cannot control its host and therefore has no economic importance (BRIDWELL, 1918; TEICHMANN & ANDRES, 1920; GOODRICH, 1921). Anyone who has had an accidental infection of *Choetospila* in a grain weevil culture will strongly doubt at least the first contention. LOOSJES (1957) seems to be of the opinion that *Choetospila* may be of some significance in the control of the grain weevil, but as yet no practical control measures can be suggested with the use of *Ch. elegans.*

By soaking wheat in water, or better still in 70% alcohol the kernel becomes soft and the fauna inside can be readily studied. The parasite is an ectoparasite and is situated generally an the dorsal side of the host larva. We never found more than one parasite per host larva. When the parasite larva had pupated the host had been exploited to such an extent that it could not continue its development. Only in one case out of many hundreds a host pupa and a parasite pupa were found side by side in one kernel.

WHICH STAGES OF THE HOST CAN BE PARASITIZED EFFECTIVELY

If we take a sample of wheat in which we have allowed a number of weevils to oviposit for one day, we know approximately at what stage of development the host will be on any following day. If we then expose part of such a sample to the parasite, also for one day, we can ascertain whether the host at the given stage could be parasitized effectively.

We used test tubes each containing 40 wheat kernels in which 10 ♀♀, together with 5 ♂♂, of *S. granarius* had oviposited on some previous day and to which were added 5 ♀♀ of the parasite at the required date, during one day. These test tubes were kept until no more insects emerged. For each age of the host 5—10 tubes were used. The controls yielded a mean of about 20 weevils per test tube, but the variation was large.

The wheat containing 1—9 days old *Sitophilus* never gave rise to a parasite and the adult weevils appeared in due course. Weevils 10 days old gave one parasite in one tube out of 7, and a normal number of weevils in all tubes.

The 11 day old stage produced 7 parasites, and the stages from 12 to 30 days old gave numbers varying from 1 to 15 per test tube and a mean of nearly 7 parasites per tube.

When the weevils were older than 33 days, again no parasites emerged, and the weevils developed normally.

If we compare these periods with the table giving the development of *Sitophilus* (Table I) we see clearly that eggs and 1st stage larvae are not parasitized effectively. As the 11 and 12 day old specimens only yield few parasites the 2nd stage larva apparently is also not entirely satisfactory as host.

Again the oldest specimens gave no parasites. This does not coincide well with the mean age at which pupation occurs. We must remember, however, that there is considerable variation in the duration of the development and that many specimens will not have reached the pupal stage on the 32nd day. From Table II we see that maximum emergence occurs on the 37th day. According to Table I this would mean the end of the fourth larval stage on the 28th day. But a considerable number of adults emerge up till the 41st day. These will have pupated on the 32nd day. After the 43rd day only very few adults emerge. The coincides very well with the absence of parasites in tubes with hosts exposed to parasitation when they were older than 33 days. We therefore believe that the conclusion is warranted that pupae are no adequate host for this parasite.

The numbers of weevils are too low for accurate determinations of the mean effectiveness of parasitation, but we can estimate an approximate percentage if we take the number of emerging weevils + parasites as the number of hosts originally present and the number of parasites as effective parasitation. The data thus obtained are given in Fig. 1.

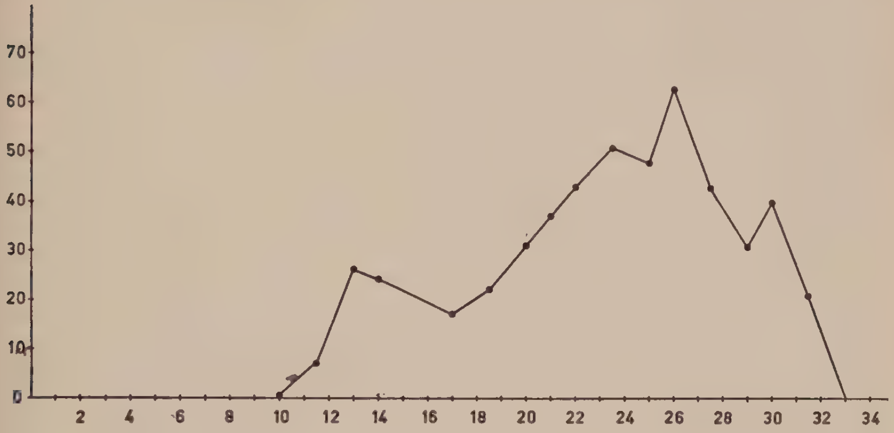

Fig. 1. Abscissa: age in days of weevils. Ordinate: percentage "effective parasitation".

This shows that the age of about 20—30 days of the host yields the highest percentage of parasites.

HOST FINDING IN CHOETOSPILA

As the larvae of *Sitophilus* are normally found inside the wheat kernel the parasite must be able to find the kernel in which the larva lives and must find the larva in the kernel to oviposit on the larva or at least near it. We may suppose that the parasite larva is unable to migrate through the endosperm of the wheat in search of its host.

We first tried to establish if there is any preference for infested wheat as compared with wheat containing no weevils.

In a one litre jar, three wide necked bottles were placed, one with 50 cc fresh wheat, one with the same amount to which about 500 adult weevils were added and one with 50 cc infested wheat containing large numbers of all developmental stages of the weevil, but no adults. In the centre of the jar between the bottles a small open vial was placed containing a number of adult parasites, lightly anaesthetised with carbondioxide. On recovery they could move about freely in the jar and enter the bottles. One experiment was done with all the bottles containing only fresh wheat. The results are given in Table III.

TABLE III

HOST FINDING IN CHOETOSPILA

duration of experiment	2 hrs		6 hrs		12 hrs	
numbers of parasites	155 ♀ 94 ♂		38 ♀ 14 ♂		31 ♀ 9 ♂	
numbers in bottles	90 ♀ 6 ♂	58% 7%	35 ♀ 7 ♂	92% 50%	30 ♀ 6 ♂	97% 67%
fresh wheat	12 ♀	8%	5 ♀	13%	1 ♀	3%
wheat + weevils	21 ♀	13%	8 ♀	21%	4 ♀	13%
wheat + preadult stages	57 ♀	37%	22 ♀	58%	25 ♀	81%

duration of experiment	6 hrs	
number of parasites	100 ♀ 52 ♂	
number in bottles	40 ♀ 17 ♂	40% 34%
fresh wheat	15 ♀	15%
fresh wheat	9 ♀	9%
fresh wheat	16 ♀	16%

These results show that the female parasites can react to certain stimuli from preadult stages of the weevils. There is a slight indication that wheat with adults is more attractive than pure wheat. It should be noted that the longer the experiment lasts the clearer the preference for the bottle with the preadult stages becomes, and that the number of parasites in the bottles with the adults is reduced about as much as in the fresh wheat. Once the bottle with the preadult stages has been found, the parasites apparently tend to remain there. No conclusion can be reached from these data on either the kind of stimulus which leads the parasites to their goal or the distance from which it can be perceived.

As a next step to finding the stimuli which lead up to the oviposition of the female, we observed a number of females in a petridish in which we had placed some grains which were either free from weevils or contained larval stages I or IV. It was soon obvious that the female parasites showed a great deal more interest in the grains with larva IV than in any other. The time spent on the kernels was longer and also their behaviour was different. When searching, the wasps continuously vibrated their antennae. On the grains containing IVth stage larvae this was intensified to what we came to call drumming on the grain.

TABLE IV

ACTIVITIES OF CHOETOSPILA IN A THREE HOUR PERIOD

time spent on empty grains	10 min	50 sec
id on grains with L I	9 .	40 .
id id L IV	50 .	16 .
id elsewhere and other activities	107 .	25 .

In one period of nearly three hours during which the activities of a female were accurately timed we found the following differences in behaviour (Table IV).

As the time spent on one grain increases, the drumming is gradually concentrated on a small area of the grain and after some time the wasp turns round and oviposits in or near this area. When the grain is then opened the larva is always to be found near the surface where oviposition has taken place.

Clearly the larva is found within the grain by means of a sense which is located in the antennae.

Free larvae are never attacked. They are inspected but only for a short time. The releasing situation for oviposition activity apparently is "larva in grain". In one case, owing to damage of the grain, a larva was partly uncovered. The inspection by the parasite proceeded normally with continuous drumming on that part of the grain which still covered the larva. No particular attention was given to the uncovered part of the larva. Finally the parasite turned round for the oviposition and misjudging the distances sunk its ovipositor directly into the uncovered part of the larva. In spite of this direct parasitation, the previous behaviour of the parasite is further proof of the fact that the "larva in grain" is the object sought for.

The fact that a free larva is not attacked and that oviposition behaviour proceeds normally also when the larva is partly uncovered rules out the possibility that it is an olfactory stimulus from the larva which induces oviposition. As we can exclude optical or temperature stimuli the only remaining sense is mechanical. That is to say, either tactical or acoustical stimuli lead the wasp to its host.

It is known that acoustical stimuli play a great part in the finding of the host larva (in wood) for certain hymenopterous parasites (cf PUMPHREY, 1950). Leaving aside the difference between feeling and hearing of vibrations, a point on which some discussion is still going on, but which is not essential to this investigation, we have finally two further observations to substantiate our conclusion.

A partly hollow grain, without a larva, was being inspected on its hollow underside by two parasite adults. A third parasite walked onto the grain and inspected it, drumming vigorously, and finally sunk its ovipositor several time into the grain, the first two animals still occupying the space in the grain. Apparently this situation fulfilled all the requirements necessary for the oviposition reaction. We do not know whether an egg was actually laid. It is quite possible that contact of the tip of the ovipositor with the larva is neccessary for this last phase of the total complex of reactions called "ovipositon".

The same situation was created on purpose a second time and again the parasite introduced its ovipositor into the grain which harboured only adults of its own kind.

The stimulus in this situation cannot have been anything else but mechanical, calling it tactical or acoustical being mainly a question of definition.

This would agree well with the observation that eggs and probably pupae are not parasitized as they produce no vibrations. The first and second stage larvae perhaps do not produce the right kind of vibrations or, more probably, produce vibrations of insufficient intensity to be detectible by the parasite.

Whether the slight preference of female parasites for the bottle with adult weevils is also caused by vibrations set up as these adults move about among the wheat is one of the many problems which await further investigation.

ZUSAMMENFASSUNG

Der Parasit *Choetospila elegans* lebt als Ektoparasit an Käferlarven und wurde in Beziehung zu *Sitophilus granarius* untersucht. In der Regel wird nur das 3. und 4. Larvenstadium parasitiert, aber es ist möglich, daß gelegentlich ältere Larven des 2. Stadiums befallen werden.

Die innerhalb der Körner lebenden Larven werden durch Wahrnehmung mechanischer Reize gefunden, die durch Aktivität der Larven hervorgerufen, aber auch von anderen Tieren nachgeahmt werden können, die sich innerhalb eines hohlen Kornes bewegen. Das stimmt mit der Tatsache überein, daß die Parasiten die Körner mit den größten der aktiven Stadien des Käfers auswählen.

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ÜBER DIE URSACHEN DER UNTERSCHIEDLICHEN RESISTENZ VON *VICIA FABA* L. GEGENÜBER DER BOHNENBLATTLAUS, *APHIS (DORALIS) FABAE* SCOP.

V. ANTIBIOTISCHE WIRKUNGEN AUF DIE VERMEHRUNGSKRAFT [1])

VON

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Landwirtschaftswissenschaften zu Berlin

An Primärblättern junger Keimpflanzen von Rastatter und Schlanstedter Ackerbohnen (*Vicia faba*) zeigt sich ein antibiotischer Resistenzunterschied in der parthenogenetischen Vermehrungsrate und -geschwindigkeit der Schwarzen Bohnenlaus, *Aphis fabae*, der auf abgeschnittenen Blättern nahezu völlig verschwindet. Dies steht vermutlich mit dem wechselnden Nährstoffgehalt in ihren Phloemen in Beziehung.

Bei der ersten eingehenderen kausalen Analyse des verschieden starken Befalls von Acker- und Gartenbohnen durch die Schwarze Bohnenlaus war DAVIDSON (1922, 1925) aufgrund unterschiedlicher Vermehrungsraten der Aphiden auf den geprüften Sorten zu der Überzeugung gelangt, daß ihr Resistenzgrad von ihrer ernährungsphysiologischen Eignung für die Blattläuse bedingt sei. Er benutzte dabei die pro Zeiteinheit produzierte Nachkommenzahl einzelner Mutterläuse als Maßstab für diese antibiotische Resistenz. Schwierigkeiten, ähnliche reproduzierbare Serien gestufter Resistenz in anderen Sortimenten nachzuweisen und der Befund, daß selbst zwischen den extremsten Gliedern einer solchen Reihe, wie der hochanfälligen Schlanstedter (S) und der schwach befallenen Rastatter (R), die Unterschiede in der Vermehrungsrate der Läuse offensichtlich zu gering waren, um die so verschieden starke Feldanfälligkeit der Bohnen zu verstehen, führten zunächst zur Suche nach einem anderen Resistenzmechanismus.

In der Tat konnte bei entsprechenden ausgedehnten Freilanduntersuchungen eine sehr auffällige Präferenzresistenz nachgewiesen werden (H. J. MÜLLER 1951, 1953). Der jeweils innerhalb von 24 Stunden durch Zuflug fundatrigener und (oder virginogener Geflügelter entstehende Initialbefall ist auf der anfälligen Schlanstedter konstant drei- bis fünfmal größer als auf der resistenten Rastatter. Bei gleicher Anflugquote auf beide Sorten entwickelt sich der Unterschied aufgrund bislang unaufgeklärter Wahlakte der gelandeten Bohnenläuse, indem sich auf den Schlanstedter Pflanzen etwa zehnmal mehr endgültig ansiedeln als auf den Rastattern, während die überwiegende Mehrzahl von beiden Sorten wieder abfliegt.

Neben den Untersuchungen zur Aufklärung des Wirtswahlverhaltens der

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[1]) Quedlinburger Beiträge zur Züchtungsforschung Nr. 38.

Bohnenläuse wurde jedoch weiter versucht, die antibiotischen Effekte der Pflanzen auf das Vermehrungspotential der Läuse zu erfassen. Dazu wurden je 5 Linien eines von einer einzigen Fundatrix abstammenden Klons in ununterbrochener Generationenfolge auf abgeschnittenen und in Wasser eingefrischten Blättern der beiden Sorten in der Form gezogen, daß jeweils die zuerst geborene Tochter nach Erlangung der Geschlechtsreife auf ein neues Blatt übertragen wurde.

Diese Methode der „Erstgeborenenzuchten" hat gegenüber dem DAVIDSON'schen Zuchtverfahren auf ganzen Pflanzen und innerhalb willkürlich festgesetzter, längerer Zeitintervalle folgende Vorteile: Die Nachkommen der Mutterlaus können sich nicht auf der ganzen Pflanze zerstreuen und in der Beobachtung unzugänglichen Blattwinkeln und Jungtrieben der genauen Zählkontrolle entziehen. Verwechselungen und Überschneidungen mit der parthenogenetischen Nachkommen-produktion der Töchter und Enkelinnen, die von der Wüchsigkeit der Pflanzen, der einzelnen besiedelten Organe, von Übervölkerungserscheinungen, Mikroklima usw. sehr unterschiedlich beeinflußt werden und die Ergebnisse leicht verfälschen, fallen fort. Infolgedessen kann sowohl der Zeitpunkt der Häutung der erstgeborenen Tochter zur Imago wie auch die Anzahl ihrer bis dahin in der Folge geborenen Geschwister genau festgestellt werden. Die dadurch mögliche Benutzung eines biologisch sinnvollen Zeitintervalls, nämlich der Zeitspanne von der Reifehäutung der Mutter bis zur Reife ihrer ersten Tochter, gestattet die exakte Ermittlung der pro Zeiteinheit höchstmöglichen Generationenzahl (Erstgeborenenzucht) sowie der pro Erstgeborenengeneration erzeugten Nachkommenzahl. Die fortlaufende Zucht über viele Generationen — im Winter bei Zusatzbeleuchtung (16—18 Std Licht) und ca 20° C — muß außerdem zu einer Summierung und statistischen Sicherung der im einzelnen vielleicht zu geringfügigen Unterschiede führen.

Wie bereits an anderer Stelle kurz mitgeteilt (H. J. MÜLLER 1956), entstanden bei diesem Verfahren von 1948 bis 1956 während insgesamt 2925 Tagen auf Einzelblättern der Schlanstedter Ackerbohnen (S) 387, auf den Blättern der Rastatter Ackerbohnen (R) 381 parthenogenetische Generationen. Der Unterschied in der Entwicklungsgeschwindigkeit ist also so gering, daß die Schlanstedter Serie der Rastatter erst nach ca 50 Generationen — unter den angegebenen Gewächshausbedingungen also erst nach ca einem Jahre — um eine Generation vorausgeeilt ist. Der Unterschied in der Reproduktionsrate ist mit 8,5% zwar etwas größer, aber auch gering. Die auf den S-Blättern lebenden Jungfern erzeugten bis zur Reife ihrer erstgeborenen Tochter im Mittel 46,9 Töchter, die auf R-Blättern sitzenden dagegen mit nur 42,9 also vier Töchter weniger.

Die Beurteilung dieser Befunde wird durch den einzigen, aber schwerwiegenden Nachteil der Methode erschwert, daß sich die Blattläuse dabei nicht an intakten Pflanzen, sondern an isolierten Blättern entwickeln. Selbst wenn dazu — wie in unseren Versuchen — nur Blätter gleicher Internodien, gleich alter und gleich vorbehandelter Pflanzen verwendet werden, so müssen sich diese Blätter (— meist 2.—3. Primärblatt —) während des Aufwachsens der jeweiligen Tochterfamilie physiologisch anders entwickeln als an der Pflanze. Wenn auch der Ablauf einer Erstgeborenengeneration durchschnittlich innerhalb von 8—9 Tagen vollendet ist, die Blätter aber erst nach 10—14 Tagen Verfärbungen und später Welkeerscheinungen zeigen, so unterliegen sie doch zweifellos schon vorher Abbau- und Verfallsprozessen, die mit einem beschleunigten Alterungsvorgang zu vergleichen sind, da der normale Konnex mit der bewurzelten Pflanze fehlt. Insbesondere im Hinblick auf die Erkenntnisse KENNEDYS und seiner Mitarbeiter (1950, 1951, 1953, 1958) über den ernährungsphysiologischen Wert junger, reifer und alternder Blätter für Aphiden, war zu bezweifeln, ob das geschilderte Verfahren die normalen Verhältnisse fehlerfrei widerspiegeln und etwaige antibiotische Resistenzeffekte richtig zum Ausdruck bringen könnte. Ein Vergleich mit ähnlichen Aufzuchten auf Blättern intakter Pflanzen erschien deshalb dringend notwendig.

MATERIAL UND METHODEN

Zu diesem Zweck wurden im Sommer 1957 zwei parallele „Erstgeborenen"-Zuchtserien mit je 5 Linien des gleichen *Aphis fabae*-Klons (Fundatrix in Frühjahr 1948 von *Evonymus europaeus* in Quedlinburg entnommen) durchgeführt: die eine in der oben geschilderten Weise an abgeschnittenen, in Wasser eingefrischten Primärblättern (Stielgläschen-Methode, s. H. J. MÜLLER 1955) der beiden Sorten Rastatter und Schlanstedter (je 5 Parallelserien) und die andere auf entsprechenden Blättern bewurzelter eingetopfter Jungpflanzen beider Sorten (ebenfalls je 5 Parallelserien). Als Ausgangsmaterial dienten im Gewächshaus bei 15—20° C und Langtag angezogene Pflanzen, sobald sich ihr erstes Primärblatt voll entfaltet hatte, d.h. wenn sich das zweite in der Entfaltung und das dritte noch in mehr oder weniger gerolltem Zustand befand. In beiden Zuchtserien diente jeweils nur das erste Primärblatt und zwar immer nur das eine Fiederblatt als Substrat für die Blattlauskultur. Die schuppenförmigen Niederblätter, das nicht benutzte Fiederblatt des ersten Primärblattes, der restliche Haupttrieb über dem ersten Primärblatt sowie bei den Topfpflanzen alle etwa noch erscheinenden Ersatztriebe wurden abgeschnitten, so daß sich bis auf die Bewurzelung das Pflanzenmaterial in beiden Serien weitgehend glich (s. Abb. 1). Die Stielgläschen

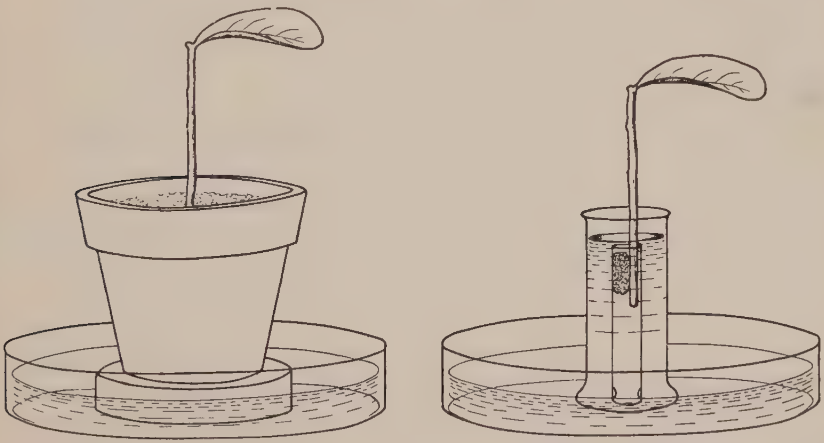

Abb. 1. Schematische Darstellung der zur Aufzucht verwandten, bis auf die eine Hälfte des ersten Primärblattes beschnittenen Jungpflanzen von *Vicia faba*. Links: normal, in Erde wurzelnd. Rechts: abgeschnitten und in Leitungswasser eingestellt.

mit den abgeschnittenen Blättern sowie die Topfpflanzen standen zur Sicherung gegen Ab- und Zuwanderung von Blattläusen in wassergefüllten Schalen. Zu Vermeidung stauender Nässe waren die Blumentöpfe im unteren Teil mit einer Scherbenpackung versehen. Blätter und Pflanzen standen während des Versuchs auf einer Gewächshaustablette bei ca 20° C und erhielten im Frühjahr und Herbst Zusatzbeleuchtung durch Leuchtstoffröhren (Langtag mit 16—18 Std Licht). Der Versuch wurde in den letzten Apriltagen (28.4) angesetzt und nach einem halben Jahr (187 Tagen) am 31. Oktober abgebrochen. Für die S-Blätter und S-Pflanzen dienten Tiere der seit 1948 auf S-Blättern, für R-Blätter und R-Pflanzen die auf R-Blättern gehaltenen Linien des erwähnten Klons als Ausgangsmaterial.

Alle 20 Zuchten (5 S-Pflanzen, 5 R-Pflanzen, 5 S-Blätter, 5 R-Blätter) wurden täglich wenigstens dreimal kontrolliert, anfangs um die manchmal herabfallenden Muttertiere wieder auf das Blatt zu setzen, später vor allen, um den Zeitpunkt der letzten Häutung der erstgeborenen Tochter zu erfassen. Diese wurde nach Aushärtung ihrer Kutikula mit einem Kamelhaarpinsel auf ein neues Blatt übertragen. Das alte Blatt wurde nach letzter Auszählung aller nachgeborenen Geschwister der Erstgeborenen mitsamt ihrer Mutter vernichtet.

Für die gewissenhafte Betreuung der Zuchten danke ich meiner Mitarbeiterin Fräulein E. Knorr herzlich.

ERGEBNISSE

Im Laufe der reichlich sechsmonatigen Gesamtversuchszeit (187 Tage) entwickelten sich auf den Blättern im Mittel der zehn Linien genau 23, auf den Pflanzen dagegen nur 19,3 Generationen, also mehr als 3½ Generationen weniger. Die Entwicklungsgeschwindigkeit erscheint also auf den Pflanzen deutlich, um fast 20%, geringer als auf den abgeschnittenen Blättern. Dabei ist der Unterschied zwischen den beiden Sorten auf den Blättern (R = 22,8 und S = 23,2) mit 0,4 Generationen geringfügig, auf den Pflanzen dagegen (R = 18,6 und S = 20,0) mit fast 1½ Generationen mehr als dreimal so groß; d.h. auf den bewurzelten Pflanzen kommt der Sortenunterschied viel deutlicher zum Ausdruck. Der auf Blättern in einem halben Jahre ermittelte Vorsprung der S-Zuchten von knapp einer halben Generation entspricht gut der in der Dauerkultur (s.o. S. 183) erwähnten von einer ganzen pro Jahr.

Entsprechend der Generationenzahl ist umgekehrt die Generationsdauer auf den Blättern mit im Mittel 8,0 Tagen kürzer als auf den Pflanzen, wo sie mit 9,1 Tagen um mehr als einen Tag länger währt. Auch hier tritt der Unterschied zwischen den beiden Sorten in den Blattzuchten kaum in Erscheinung (R = 8,1, S = 7,9 Tage), während er in den Pflanzenzuchten mit fast einem halben Tag (R = 9,3, S = 8,9 Tage) doppelt so groß ist.

Die Größe der Nachkommenproduktion in den vier Zuchtgruppen läßt sich schon beurteilen, wenn die Gesamtzahl aller im Lauf der sechs Monate erzeugten Töchter berechnet wird. Dabei sind auch die Familien einbezogen, bei denen die Mutter vor der Reife der ersten Tochter starb (s.u.S. 186) oder sonstwie verunglückte, so daß ihre Jungenproduktion vorzeitig erlosch. Auf den Blättern wurden so insgesamt 9493 (= 62%) der Nachkommen, auf den Pflanzen dagegen nur 5818 (= 38%) hervorgebracht, d.h. — anders ausgedrückt — fast die Hälfte weniger als bei Ernährung auf abgeschnittenen Blättern. Auch hierbei ergibt sich nur ein geringer Unterschied von 7,0% zwischen den Blättern der beiden Sorten, indem sich auf R 4411, auf S 5082 Nachkommen entwickelten. Dagegen trugen die R-Pflanzen mit 2215 Läusen gegenüber 3603 auf den S-Pflanzen 24% weniger Nachkommen. Der Sortenunterschied in der Nachkommenproduktion des Gesamtversuchs wird demnach auf den Pflanzen 3½ mal deutlicher als auf den abgeschnittenen Blättern.

Für die exakte Beurteilung der Reproduktionsrate erschien es notwendig, alle diejenigen Familien von der Auswertung auszuschließen, bei denen die Mutter nicht wenigstens bis zum Tage der letzten Häutung ihrer zuerst geborenen Tochter gelebt und Junglarven abgesetzt hatte, weil die normale pro Erstgeborenen-Generation produzierte Nachkommenzahl dann natürlich nicht erreicht werden konnte.

Der Anteil dieser unvollendeten Familien an der Gesamtzahl der Familien zeigt ebenfalls sehr charakteristische Unterschiede. Er beträgt auf den Blättern im Mittel 8,75%, bei den Pflanzen dagegen 22,75% der erzeugten Generationen, also über 2½ mal so viel. In beiden Fällen sind die unvollkommen entwickelten Familien auf R häufiger als auf S und zwar tritt der Unterschied auf den Pflanzen wieder bedeutend schärfer hervor als auf den Blättern. Während bei diesen auf R 12% und auf S 7,5% der Mütter nicht bis zur Geschlechtsreife der ersten Tochter lebten, waren es auf den Pflanzen an R 57% und auf S 13,8%. Während also die Ausfälle prozentual auf den Blättern auf R nur etwa ½ mal höher lagen als auf S, betrugen sie an den Pflanzen das Vierfache.

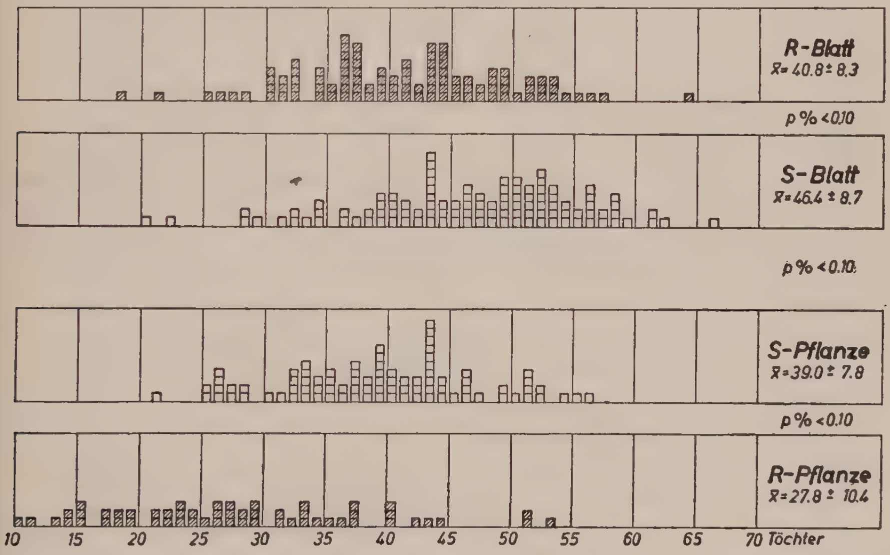

Abb. 2. Häufigkeitsverteilung der auf abgeschnittenen Blättern und bewurzelten Pflanzen von Rastatter (R) und Schlanstedter (S) Ackerbohnen gezogenen Erstgeborenen-Familien von *Aphis fabae* nach der Anzahl ihrer zwischen Geburt und Reife der erstgeborenen Tochter produzierten weiteren Töchter.

Nach Ausschluß der durch vorzeitigen Ausfall der Mutterlaus reduzierten Familien ergeben sich für die Anzahl der pro Erstgeborenen-Generation erzeugten Töchter die in Abb. 2 dargestellten Häufigkeitsverteilungen. Trotz sehr großer Streuung der Einzelwerte, läßt sich sofort erkennen, daß die Produktionsrate auf den abgeschnittenen Blättern mit im Mittel 43,6 pro Generation größer ist als auf den Pflanzen, wo sie im Mittel nur 33,4 erreicht. Dabei werden auf den S-Blättern ($\overline{x} = 46,4 \pm 8,7$) pro Generation zwar — statistisch signifikant — mehr Junglarven erzeugt als auf den R-Blättern ($\overline{x} = 40,8 \pm 8,3$). Der Unterschied ist aber relativ gering, d.h. es werden auf R nur etwa 5,6 = 12% Töchter weniger hervorgebracht als auf S. In den oben erwähnten Dauerzuchten auf abgeschnittenen Blättern war der Unterschied sogar noch geringer. Demgegenüber tritt der Unterschied zwischen den beiden Sorten in der Reproduktionsrate auf den Pflanzen viel deutlicher hervor, indem auf der R im Mittel nur 27,8 ± 10.4, auf der S dagegen 39,0 ± 7,8 Töchter pro Generation erzeugt wurden, d.h. auf R ca 29% Nachkommen weniger.

DISKUSSION

Bereits 1951 hat ARENZ in einigen weniger umfangreichen Versuchen bei künstlichem Besatz der Kartoffelsorte Ackersegen mit der Grünen Pfirsichblattlaus, *Myzus persicae,* festgestellt, daß die Vermehrungsquote der Blattläuse auf abgeschnittenen Blättern sehr viel größer, die Absterbequote dagegen geringer ist als auf intakten Pflanzen, die praktisch überhaupt keine Vermehrung gestatteten. Er vermutet als Ursache dieser Unterschiede das Freiwerden organischer Stoffe (Eiweißbruchstücke, Kohlehydrate) im abgeschnittenen Blatt als ernährungsbegünstigenden Faktor. Bei unserer über ein halbes Jahr in ununterbrochener Folge und fünffacher Wiederholung (d.h. je 5 Parallelzuchten) durchgeführten Aufzucht von Erstgeborenen-Familien der Schwarzen Bohnenlaus treten ähnliche, statistisch gesicherte Unterschiede in Reproduktionsrate und -geschwindigkeit auf: bei der Haltung auf abgeschnittenen Primärblättern einerseits und auf entsprechenden Blättern bewurzelter Jungpflanzen andererseits.

Im Anschluß an die von KENNEDY und seinen Mitarbeitern entwickelten Vorstellungen liegt es nahe, die höhere Entwicklungsgeschwindigkeit (mittlere Generationsdauer um ca 10% verkürzt) und größere Reproduktionsrate (durchschnittlich 23% mehr Nachkommen pro Erstgeborenenzucht) auf den abgeschnittenen Blättern damit zu erklären, daß das abgeschnittene Blatt während der Zeit, in der sich die Erstgeborenen-Familie auf ihm entwickelt (8—10 Tage), einem viel rascheren Alterungsprozeß unterliegt als im natürlichen Verband der bewurzelten Jungpflanze. Da in alternden Blättern bekanntlich eine Mobilisierung der Gewebe-Eiweiße und ein erhöhter Abtransport löslicher Eiweißbausteine in den Gefäßen stattfindet, muß der Nährwert des von den Bohnenläusen aus abgeschnittenen Blättern aufgenommenen Phloemsaftes höher sein als in solchem aus intakten Blättern, die während der Entwicklung der Kolonie weitgehend in vollreifem, vorwiegend assimilatorischem Zustand verharren. Dabei ist erhöhter N-Gehalt der Nahrung natürlich für den Aufbau körpereigener Eiweiße bei der Produktion von Nachkommen von ausschlaggebender Bedeutung. Mit dem fallenden Aminosäurespiegel in den Gefäßen des reifen Laubes wird von KENNEDY das Abwandern wirtswechselnder Aphiden von ihren holzigen Primärwirten nach Beendigung des Frühjahrstriebes begründet, wie umgekehrt ihre Rückkehr im Herbst mit seinem Wiederanstieg vor dem herbstlichen Laubfall. Wenn sich KENNEDY's Hypothese auch in erster Linie auf die Wahl geeigneter Nahrungsquellen und entsprechende Verhaltensakte bezieht, so dürften Unterschiede im Gehalt löslicher Eiweißspaltprodukte für die Produktion von Nachkommen noch weit entscheidender sein, da bei der Nahrungswahl das Mitwirken sekundärer Pflanzenstoffe als Signalreize wahrscheinlich eine erhebliche Rolle spielt.

MITTLER's Untersuchungen an *Tuberolachnus salignus* haben die Bedeutung des osmotischen Druckes im Phloemsaft für die Nahrungsaufnahme der Aphiden deutlich gemacht. Ob er in bewurzelten einblättrigen Jungpflanzen größer oder geringer ist als in abgeschnittenen Primärblättern, ist bislang unbekannt. Offensichtlich ist er aber in keinem Fall so gering, um das Abwandern von den Blättern zu verursachen. Daß er am Zustandekommen der festgestellten Vermehrungsunterschiede der Aphiden beteiligt ist, erscheint zweifelhaft. Er müßte dann in abgeschnittenen Blättern größer sein als in Blättern bewurzelter Pflanzen. Dies würde mit der oben geforderten höheren Konzentration N-reicher Assimilate in alternden Blättern in Einklang stehen.

Für die Resistenzphysiologie ist unter den vorliegenden Ergebnissen die Tatsache am überraschendsten, daß der antibiotische Effekt der Sorten in seiner Wirkung auf das Vermehrungspotential ungeflügelter Jungfern der Bohnenlaus in ganz verschieden starkem Maße zum Ausdruck kommt. Während er auf abgeschnittenen Blättern zwar statistisch signifikant nachweisbar ist, indem auf den Rastattern im Mittel 5,6 (= 12%) Jungfern pro Erstgeborenen-Generation weniger produziert werden, so ist der Unterschied im Vergleich mit dem auf den Primärblättern bewurzelter Pflanzen auftretenden jedoch gering, wo im Mittel auf S 39,0, auf R dagegen nur 27,8 Töchter pro Generation hervorgebracht werden, also um 29%, d.h. fast ein Drittel weniger. Diese Vervielfachung des Resistenzunterschiedes zwischen R und S auf bewurzelten Jungpflanzen gegenüber dem auf isolierten Blättern kommt jedoch nicht nur in der Reproduktionsrate, sondern auch in der Entwicklungsgeschwindigkeit (Generationenzahl und -dauer) sowie in der Absterberate der Mütter und demzufolge auch in der Gesamtmasse der produzierten Nachkommen deutlich zum Ausdruck (s.o.S. 185 ff).

Hält man die oben gegebene Interpretation der Vermehrungsunterschiede der Bohnenläuse auf isolierten und bewurzelten Primärblättern für zutreffend, so bietet sich als Erklärung für die gleichzeitig damit auftretende Differenz des antibiotischen Wirkungsgrades zweier Ackerbohnensorten folgende Weiterführung der Hypothese an: Auf reifen Blättern normaler, bewurzelter Rastatter Pflanzen ist die Vermehrungspotenz der Schwarzen Bohnenläuse infolge der zu geringen Menge irgendwelcher essentieller Nahrungsstoffe, wahrscheinlich gewisser Aminosäuren, viel geringer als auf entsprechenden Blättern Schlanstedter Ackerbohnen. In den Leitbündeln abgeschnittener Blätter dagegen sind auch diese für den Baustoffwechsel der Bohnenlaus notwendigen Eiweißbausteine infolge des Eiweißabbaus im alternden Blattgewebe selbst in Rastatter Blättern in so ausreichend hoher Konzentration vorhanden, daß ihre Vermehrungsrate und -geschwindigkeit die der auf S-Blättern lebenden Läuse fast erreicht, jene der auf S-Pflanzen ernährten sogar — statistisch signifikant — etwas übertrifft ! Zwar führt die Ernährung auf abgeschnittenen Blättern auch bei den auf S lebenden Bohnenläusen zu einer statistisch signifikanten Steigerung der Vermehrungsquote, doch bleibt sie relativ gering, weil die Konzentration des oder der lebensnotwendigen Nährstoffe (Aminosäuren) schon auf den intakten Pflanzen offenbar fast optimal ist, so daß ihre weitere Erhöhung in abgeschnittenen Blättern nur noch eine schwache Wirkung auf die Vermehrungsrate usw. ausübt. Eine entsprechende chemische Analyse isolierter Blätter und normal bewurzelter Jungpflanzen beider Sorten ist in Zusammenarbeit mit der Chemischen Abteilung des Instituts für Pflanzenzüchtung Quedlinburg (Leiter Dr. W. MATTHIAS) in Angriff genommen.

Der große, im Feldanbau in Erscheinung tretende Unterschied im Blattlausbefall der beiden Ackerbohnensorten Schlanstedter und Rastatter, d.h. die starke Anfälligkeit der ersteren und die meist nur sehr schwache Besiedlung der letzteren, beruht nach unseren früheren (H. J. MÜLLER 1951, 1953) und den nun vorliegenden Untersuchungen auf mindestens zwei grundverschiedenen Resistenzmechanismen: einmal auf einer deutlich ausgeprägten Präferenzresistenz und zum anderen auf einer weniger leicht erkennbaren Antibiosis. Die Präferenzresistenz kommt in der drei- bis fünffach stärkeren Bevorzugung der Schlanstedter bei der zur Initialbesiedlung führenden Wirtswahl der fundatrigenen oder virginogenen Geflügelten zum Ausdruck und zwar unabhangig von Art und Alter der gebotenen Organe der beiden Sorten (H. J. MÜLLER 1953, KENNEDY 1958). Welche Eigenschaften der Pflanze bei dieser Wirtswahl für die Blattlaus entscheidend sind, ist noch völlig ungeklärt, doch kommen Duftreize höchstwahrscheinlich nicht in Betracht, da die Wahl erst nach der Landung, also nach direktem Kontakt mit der Pflanze durchgeführt wird und demzufolge vermutlich taktile Reize bzw. mechanische oder Geschmackswahrnehmungen bei den sogenannten kurzfristigen Probesaugstichen die entscheidende Rolle spielen dürften. Der antibiotische Resistenzeffekt der Rastatter Ackerbohnen äußert sich in einer Hemmung von Geschwindigkeit und Umfang der parthenogenetischen Fortpflanzung, die zweifellos auf einer Insuffizienz bestimmter, für den Aufbau körpereigener Eiweiße notwendiger Nährstoffe beruht; denn sie verschwindet weitgehend, wenn durch Abschneiden (und Einfrischen in Wasser) eine künstliche Alterung der ernährenden Pflanzenorgane und damit vermutlich eine Anreicherung von Eiweißbausteinen in ihren Leitungsbahnen hervorgerufen wird.

Nach KENNEDYS Vorstellungen müßte das Gleiche in jungen, wachsenden Organen eintreten. Wahrscheinlich beruhen die großen Schwankungen der Nachkommenzahlen in Resistenzprüfungen nach dem DAVIDSONschen Verfahren darauf, daß die produzierenden Mutterläuse nicht immer an ernährungsphysiologisch gleichwertigen, sondern bald mehr an jungen, bald mehr an reiferen oder alternden Geweben saugen und deshalb bei Wiederholungen sehr unterschiedliche Tochterzahlen liefern. Dies wird sich umso stärker auswirken, wenn auch die Produktion der Töchter und Enkelinnen dazukommt.

In natürlichen Feldbeständen siedeln sich die Bohnenläuse auf beiden Sorten bevorzugt an jungen Blättern, an den Blütentrieben und Stengeln und charakteristischerweise auch auf alternden, schon vergilbenden Blättern, dagegen kaum am reifen Laub an. Der Antibiosiseffekt der Rastatter dürfte sich also praktisch nur in begrenztem Unfang auswirken.

Die Beobachtung KRONENBERGS & DE FLUITERS, daß alternde, schon vergilbende Blätter blattlausresistenter Himbeersorten doch von *Amphorophora rubi* befallen werden, fügt sich den vorliegenden Untersuchungen ebenso gut ein wie der Hypothese KENNEDYS. In diesem Zusammenhange könnte die Antibiosisresistenz der Ackerbohnen gewissermaßen als eine auf bestimmte Entwicklungsstufen der Pflanzengewebe beschränkte Altersresistenz verstanden werden, wobei die reifen Blätter das relativ resistente Stadium darstellen.

Ob, wie KENNEDY meint, der Wirtswahl der Aphiden, zumindesten der temporären Präferenz bestimmer Wirtspflanzen, die gleichen Ursachen zugrunde liegen wie den auf Antibiosis beruhenden Resistenzerscheinungen, nämlich ernährungsphysiologische Bedingungen, d.h. bestimmte Entwicklungszustände der Pflanzen, ist noch unsicher. Daß beim Probesaugen sekundäre Pflanzenstoffe etwa als Geschmacksreize zur Wirkung gelangen können, erscheint allerdings ebenfalls fraglich, weil offensichtlich dabei keine Zellen verletzt werden. Daß aber die als Grundnährstoffe dienenden primären Assimilationsprodukte geschmacklich wahrgenommen werden können, erscheint zweifelhaft. In Anlehnung an MITTLERS Befunde könnte man vielleicht eher annehmen, daß ihre Konzentration über den osmotischen Druck als die Wirtswahl beeinflussender Faktor wirksam wird. Dann würde die Menge der Grundnährstoffe (etwa der Aminosäuren und der Zucker) auf physikalischem Wege die Präferenz, auf chemisch-ernährungsphysiologischem Wege die Antibiosis bedingen. Das würde jedoch voraussetzen, daß der kohlehydratreiche Phloemsaft reifer, assimilierender Blätter geringere osmotische Werte als der N-reiche junger und alternder Blätter aufweist.

Die Tatsache, daß sich der Präferenzunterschied, d.h. die Wahl der Bohnenläuse zwischen R und S an Blättern jeden Alters und Organen der verschiedensten Art immer in gleichem Verhältnis manifestiert (H. J. MÜLLER 1953), der Antibiosiseffekt auf die Reproduktion aber je nach Entwicklungszustand und Nährwert des pflanzlichen Substrats in sehr unterschiedlicher Stärke, spricht doch vorläufig sehr gegen die Annahme eines einheitlichen Prinzips für beide Resistenzmechanismen.

SUMMARY

The rate of reproduction and the velocity of development of Black Aphids, *Aphis* (*Doralis*) *fabae* Scop., are proofed on first-formed leaves (see Abb. 1 above) of Rastatter (R) and Schlanstedter (S) fieldbeans 1) by numbering the daughters, which are born by one young virginogen mother in the time between birth and maturity of her first born daughter and 2) by measuring this time (of a such a "firstborn generation") in days for many generations, which are continued by placing the young ripe first born daughter as mother of the next generation on a new leaf each time. The breedings are made during half a year in two parallel series of five lines each on R and S a) on cut and watered first-formed leaves and b) on equivalent leaves of rooted plants.

On the cut leaves in water the velocity of development, the total number of progeny, and the rate of reproduction (calculated by dividing the number of daughters in the first-born generation by the period from birth to maturity of the first-born daughter) were on

the average much greater than on rooted seedlings of the same varieties. The duration of each generation and the mortality of the mothers was significantly lower on the cut leaves.

The differences between the two varieties were only slight on cut leaves in water, but they were more conspicuous on the rooted leaves. With reference to KENNEDY's idea of the nutritional value of differently aged leaves, it is suggested that the different concentrations of soluble protein components in the phloem may be the cause of these antibiotic effects. As this concentration is probably the same in cut leaves as in ageing ones, the aphids find more food there and therefore produce more progeny more rapidly than on the mature leaves of rooted plants.

As the optimum conditions for reproduction are found on cut leaves of R, and as these seem similar to those found on S, the increase in food available apparently compensates for the differences in antibiotic resistance between the two varieties.

Some possible mechanisms for this antibiotic resistance are discussed, especially as it has formerly been stated that R beans appear to be resistant to alate aphids when these choose their host plants.

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THE ORIENTATION OF HORSE FLIES AND DEER FLIES (TABANIDAE, DIPTERA):

I. THE ATTRACTANCE OF HEAT TO TABANIDS

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Tabanid flies display an exceptionally strong tendency to orientate to and congregate in enclosures which accumulate heat during daylight. In order to study this behaviour to the best advantage and perhaps also to exploit it toward practical control a "helio-thermal" trap was designed and tested. This trap converts solar radiation to heat and is so designed that tabanids collect in a killing bottle. A number of ecological, ethological and practical applications of the trap are discussed in the light of data obtained to date.

Wherever tabanids occur in large numbers, as they do in marshy areas of Canada and elsewhere they are recognized to be the most destructive of livestock insect pests during the height of their active season. Although this period of activity is relatively short, about six weeks, it corresponds with the time of greatest productivity and quality of pastures. As tabanids in large numbers interfere with grazing at this time the nutritional deficit incurred must greatly aggravate the loss sustained by the irritant effect of the bites.

The tabanids are notoriously elusive targets for the numerous arsenal of modern insecticides. Repellents available to date provide only ephemeral relief from the attacks of these flies. The breeding areas are generally too extensive to permit larvicidal treatments. Pending more promising developments in the chemical industry, it appeared worthwhile to investigate the behavioural peculiarities of tabanids in the hope of discovering effective means to reduce their destructiveness. This practical motive led to an investigation primarily ethological.

A most striking feature of tabanid behaviour is frequently observed by biologist and layman alike when a motor vehicle is stopped in an infested area on a warm, sunny, midsummer day when horse flies soon enter the open door windows. As a vehicle cab tends to accumulate heat and becomes appreciably warmer than the outside air and there is no other available hypothesis it must be assumed that the warmth within the vehicle somehow exerts an attractive influence on horse flies in the vicinity.

Having once entered the open side windows the horse flies then congregate at the front and rear glass winds hields, suggesting that, once inside the warm interior shaded from skylight, the flies become positively phototactic.

These observations suggest that the behaviour of tabanids exhibits a peculiarity that should lend itself to exploitation through the design of an appropriate trap. A similar thought occurred to an earlier Manitoba entomologist, Norman Criddle (HEARLE, 1938), several decades ago. However, his application of it was limited to the adaptation of existing buildings by means of screens to trap horse flies that entered with the livestock.

Various types of tse-tse fly traps were compared by MORRIS & MORRIS (1949) who attributed the attractance to visual stimulation — thermal attractance was not mentioned.

Various methods of trapping and collecting tabanids have been reviewed recently by Snow, Pickard & Moore (1957). These authors observe that the most effective device for trapping horse flies is a motor vehicle cab as described previously. While this method is adequate to the purpose of the taxonomist it can hardly have any practical application nor does it lend itself to penetrating studies of tabanid ethology.

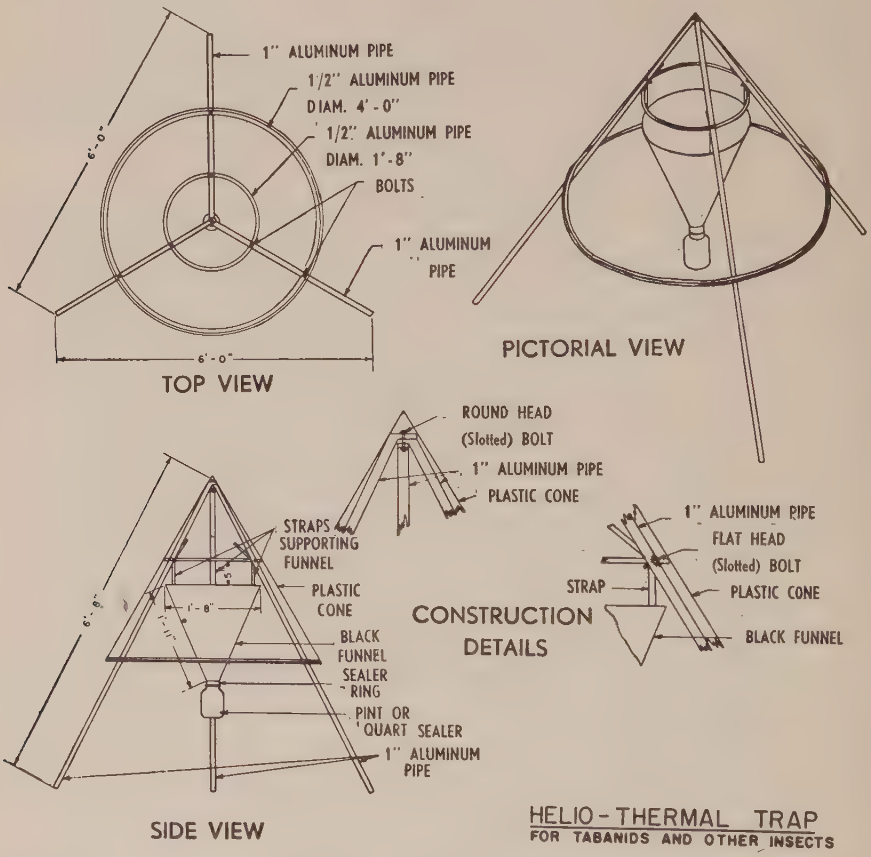

Fig. 1. Construction of the Helio-Thermal Trap

It was proposed, therefore, to design and develop a trap that would exploit the attractiveness of heat to tabanids to the best advantage. The following desiderata were taken into consideration:

1. The trap must accumulate heat below and inside the trap to initially attract the insects. This effect was obtained by the application of the greenhouse principle (i.e. a black body enclosed within a cover which is transparant to solar radiation but impedes the escape of thermal energy).
2. The trap should be so designed that, once under the trap the insects will be led

by phototaxis or otherwise to a restricted upper space where, as a consequence of thermal stress, exhaustion or contact with lethal chemicals, the flies succumb and drop through a funnel to a receiving and killing jar below (The funnel is painted black, thereby providing also the black body referred to previously).

3. The trap should be as simple as possible in design, assembled of inexpensive materials and, preferably, operate efficiently without artificial sources of energy. The essential features of the trap design are illustrated in Figure 1. The materials include aluminium tubing, nuts, bolts, translucent plastic and thin galvanized sheet metal. The plastic tent may be covered with wire mesh (not shown) to secure it against winds.

4. The trap would require protection from molestation by livestock or otherwise and this is provided by a three-sided barbed wire corral (not shown).

PERFORMANCE OF THE HELIO-THERMAL TRAP

Field trials of the trap were started early in July, 1957 when the horse fly season was well advanced at several locations north of Winnipeg where tabanid populations (mainly horse flies) were moderately heavy. The data obtained at Lake Francis are presented in Table I to illustrate the results obtained. Somewhat similar data were obtained with several other traps at three other locations but little would be gained from a presentation of the complete records, although one area yielded many more *Chrysops*. The highest average daily catch exceeded 40 horse flies, the equivalent of a serious infestation for one animal. The data do not indicate whether this is the peak capacity of a single trap since the insect population apparently was already beginning to decline.

TABLE I

Helio-Thermal Trap Captures.
Trap No. 3, Lake Francis, 1957.

Dates	*Tabanus*	*Chrysops*	Other Diptera	Other Orders
July 11—16	215	—	—	—
July 17—18	28	—	—	—
July 19—25	50	—	—	—
July 25—31	15	—	—	—
Aug. 1—7	7	3	35	—
Aug. 8—14	0	0	60 (38)	139 (3)*

Note: The open figures represent numbers of individuals; those in parentheses refer to number of species. The sign — indicates no record.
* A species of cicadellid comprised nearly all of these insects.

During the month of July, when much of the available time was devoted to constructing a series of traps with improvements in design, the insect captures were not treated in a manner to permit recovery of taxonomic data. Indeed, the potential value of the helio-thermal trap as a taxonomic tool was not appreciated until near the end of the season when the assistants were instructed to conserve such data at least to the extent that the number of species of all captures could be indicated. As Table I shows, although a considerable number of species other than tabanids are captured, at any rate late in the summer, these comprise mainly other Diptera. The only notable exception was a single cicadellid species that appeared in the trap in some numbers in the second week of August. Occasional capture of non-dipterous species occurred in the traps during the study but at present these are regarded as accidental. Inasmuch as the helio-thermal trap depends on solar radiation to generate the attractive principle it can in its present form be expected to capture only diurnally active flying insects.

As a few tabanid species are said to be nocturnal (Snow, et al 1957), these are not likely to be captured unless the trap continues to emit heat for a sufficiently long time after sundown. However, according to Philip (1931) such species apparently do not occur in Minnesota, a state adjoining Manitoba to the south. At present, neither the entomological nor the thermal data appertaining to the trap provide information relevant to this question. However, the simultaneous readings of temperatures within the trap and in the ambient atmosphere, presented in Table II, indicate the general thermal characteristics of the trap during the daylight hours. It will be noticed that the trap temperature tends to reach and remain at a level about 15° C or more higher than the ambient temperature. This trap temperature is at least equal to the upper lethal temperature for most insects. As the flies invariably congregate within the apex of the trap where it is, if anything, even warmer, they must perish unless they succumb temporarily to heat stupor. In either case, they fall through the funnel into the receiving jar below. It is advisable to charge the latter with cyanide or other non-repellent, lethal chemical to avert the possibility of recovery and escape of captured insects.

Table II

Temperature Readings for Helio-Thermal Trap No. I July 10—11, 1957.

Time of Day	Temperature within trap.	Ambient Temperature.
1.30 P.M.	40.5° C.	31.5° C.
2.00	44.1	32.6
3.30	47.0	35.0
4.30	50.0*	35.0
—	—	—
8.45 A.M.	40.0	27.0
10.00	46.0	30.0

* Maximum reading of thermometer recorded — actual temperature exceeded this by some three or four degrees.

Note: the bulb of the thermometer within the trap was located at the centre of the plane circumscribed by the upper edge of the funnel (see Figure 1).

DISCUSSION

Mode of Action of the Trap

In its present form, the helio-thermal trap was designed on the premise that heat alone is an effective attractant stimulus for tabanid flies. The observations of Philip (1931) and the success of the trap itself leave little doubt as to the validity of this assumption. This behavioural characteristic may be only a special case of the tendency of numerous insect species to accumulate in warmer spaces of the environment, observed especially in the cooler weather in the autumn. It seems probable however, that it is related more specifically to a host finding mechanism inasmuch as the typical host, for example a horse, cow, moose, or bison is a large animal with a temperature maintained somewhat above the ambient temperature even on a warm day. It is remarkable that a construction that deliberately simulates the host only in a single particular should be so effectively attractive. The way remains open however to seek and exploit other attributes of the host that may significantly increase the attractiveness of he trap. KRIJGSMAN (1930) observed such multiple effects in his study on *Stomoxys*. These possibilities are discussed in a later section.

Economic Application

The development of the helio-thermal trap as mentioned previously, was motivated initially by the hope of achieving a method of practical control of a group of serious livestock pests. Whether this objective has been achieved will be determined only through future investigation and experience. In general, traps have proved effective only in indirect ways in economic entomology. However, in the case of an insect species that, in relatively small numbers, causes very considerable damage, a really effective trap is worth considerable investigation.

A significant feature of the helio-thermal trap is that it is specific for female tabanids which, of course, is the sex that wreaks the destruction and also lays the eggs. Therefore, whether or not the practical effect is decisive in a single season, it should be accumulative, so that, over a period of two or more years, a significant reduction of tabanid populations should accrue. Tabanids are, it is true, recognized to be very strong fliers (HOCKING 1953) and one must expect immigration into the area in which traps are located. A permanent and complete eradication cannot be expected, since it is impossible to foresee a sufficient number of traps dispersed throughout the enormously extensive breeding areas of these pests. However, in view of the prospect of further improvements in the efficiency of this trap, to be outlined later in this discussion, it does not seem unduly wishful to hope for a practical application. The number of traps required to protect a pasture of a given area remains to be established and although the trap in its present form is inexpensive it is probable that it would be economical to use it only on improved pastures rather than on extensive, marginal rangelands. Again, we may recall, that when pastures are at their best, tabanids are at their worst and this is true both seasonally and through the years. Offsetting this economically awkward fact is the observation that when the tabanids are most active, the helio-thermal trap is most efficient.

Scientific Applications

Whatever may be the economic status of the helio-thermal trap it appears certain to have a number of very promising uses as an investigational tool in entomological research. The taxonomist and ecologist can obtain a large number of specimens of many species of tabanids and other Diptera through the season with little effort. The ecologist can relate the captures with climatic, edaphic and other factors. The ethologist and physiologist can study orientation and flight range in the field under the influence of a single physical stimulus (heat) not entirely unconfounded, it is true, by vision.

On the other hand, by appropriate modification of the trap, the study of tabanid behaviour can be extended so as to secure information as to the visual reactions of these insects. Indeed, we already have a very strong indication that movement greatly increases the attractiveness of the helio-thermal trap. In the course of placing traps in the field, we have observed that they begin to catch horse flies even before they are completely assembled. Apparently the physical activity of the investigators contributed considerably to the rate of capture. It is proposed to exploit this lead in 1958 by adding wind sensitive, revolving vanes. The use of painted vanes may provide information concerning colour vision. With or without this modification, helio-thermal traps will provide an objective basis for investigating the chemical sense of tabanids since they may be tested in association with either repellants or attractants. All these proposals present excellent prospects of success since they are in harmony with well known behavioural characteristics of tabanids as described for example by PHILIP (1931). These various extensions of this study are now under investigation in Manitoba.

ADDENDUM

Further observations in 1958 have revealed that in cooler seasons the helio-thermal trap as described here does not generate sufficient heat to prevent escape of captured horse flies. This was corrected by placing a transparent no-return receptacle at the apex of the trap. The black funnel is retained but it now functions only as a black body. A description of this modification will be included in a subsequent report.

RÉSUMÉ

Plusieurs espèces de tabanides semblent être fortement attirées par une source de chaleur sensiblement plus élevée que celle de la temperature ambiante. Ces espèces se comportent ainsi surtout quand il fait très chaud alors qu'elles sont tout particulièrement actives. Nous avons profité de ce comportement pour construire un piège hélio-thermique ainsi appelé parce qu'il produit une chaleur tirée de l'énergie solaire.

Les tabanides attirés par ce piège y pénètrent par la partie inférieure, et, apparemment attirés par "phototaxis" volent vers le sommet. Dans cet espace la temperature s'élève au-dessus de la limite tolérable et les tabanides sont abrutis par la chaleur. Alors ils tombent par un entonnoir dans un récipient contenant du cyanure. Les tabanides capturés sont exclusivement des femelles: fait de la plus haute importance dans l'emploi pratique de ce piège. Cependant ce piège a d'autres applications comme instrument de recherches entomologiques pour les taxonomistes, les oecologistes et les éthologues. Il est fort probable qu'un simple dispositif de moulinet actionné par le vent et créant un mouvement rotatoire augmenterait son attraction sur les tabanides et augmenterait sa valeur pratique aussi bien que sa valeur scientifique. Uu autre emploi important résulterait de son adaptation à l'étude des substances qui attirent ou repoussent ces insectes.

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UNTERSUCHUNGEN ÜBER AUFFINDEN UND WAHL DER NAHRUNG BEIM KARTOFFELKÄFER (*LEPTINOTARSA DECEMLINEATA* SAY)

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Zwecks Ausarbeitung biozönoseschonender, selektiver Bekämpfungsmethoden wurden die reflektorischen Bedingungen der Nahrungswahl des Kartoffelkäfers untersucht. Die überwinterten Käfer können im Freiland die Anwesenheit der Wirtspflanze höchstens aus einer Entfernung von einigen Dezimetern wahrnehmen. Jungkäfer sprechen nur nach erfolgter Dressur (= Nahrungsaufnahme) auf die von der Wirtspflanze stammenden olfaktorischen Reize an. Beim Auffinden der Pflanzen spielen die visuellen Reize bei den Alt- und Jungkäfern, allerdings nur aus kleinen Entfernungen, eine wichtige Rolle. Die Jungkäfer halten bei der Nahrungssuche eine konstante Laufrichtung ein. Die Frass-Stoffe sind nur begrenzt spezifisch, die Oligophagie des Kartoffelkäfers ist demnach auf eine negative Anpassung an vergällend wirkender Stimuli zurückzuführen. Die vergällende Wirkung der Stoffe ist von der chemischen Struktur weitgehend unabhängig. Eine Reduktion der Vermehrungsrate des Schädlings kann durch Veränderung des Reizstoffgehaltes der Wirtspflanze bzw. durch Anwendung von Vergällungsstoffen erreicht werden.

Es gilt für einen Gemeinplatz, dass, obzwar durch die Anwendung moderner Insektizide in der Schädlingsbekämpfung ausgezeichnete und vorher nicht geahnte Erfolge erzielt wurden, diese den Praktiker und den Theoretiker des Pflanzenschutzes vor sehr beachtenswerte Probleme stellten. Das Gros dieser Probleme ist auf die Tatsache zurückzuführen, dass die neuen Wirkstoffe ein über ihrem Anwendungsziel hinausreichendes Wirkungsspektrum besitzen, infolgedessen sie in den Kulturbiozönosen unerwünschte Änderungen hervorrufen.

Diese Gefahren, denen man noch heute nicht die gebührende Aufmerksamkeit schenkt, benötigen eine dringende Ausarbeitung *selektiver* Schädlingsbekämpfungsmethoden. Obzwar in der Erforschung selektiv wirkender Insektizide, deren Selektivität entweder auf physiologisch-toxikologischen Gegebenheiten oder auf der Anwendungsmethode beruht, wesentliche Erfolge zu verzeichnen sind, scheint es doch nicht überflüssig zu sein auch prinzipiell andere Methoden zu erforschen.

Für die Ausarbeitung biozönoseschonender Bekämpfungsmethoden bietet sich theoretisch ein Anhaltspunkt in der Tatsache, dass das Zustandekommen und die Aufrechterhaltung der Konnexe in den Biozönosen auf der spezifischen Reaktionsbasis der einzelnen Glieder fussen. Die Spezifität des Reflexmechanismus, der die tierischen Glieder der Biozönosen mit den Pflanzen bzw. miteinander zu Konnexen verbindet, macht prinzipiell die Ausarbeitung selektiver Methoden möglich, die das Entstehen oder die Aufrechterhaltung der Teilkonnexe Schädling-Kulturpflanze stören oder zerstören, ohne die Beziehungen anderer Biozönoseglieder —

z.B. die Beziehungen parasitischer Insekten zu ihren phytophagen Wirten — beeinflussen zu müssen.

Mit dieser Zielsetzung wurde im Ungarischen Forschungsinstitut für Pflanzenschutz zunächst die kausalanalytische Erforschung des Teilkonnexes Kartoffelkäfer-Wirtspflanze begonnen.

UNTERSUCHUNGSMETHODEN

Olfaktometerversuche. Zu den Olfaktometerversuchen wurde ein im Prinzip dem McIndoo'schen (1926) ähnlicher Apparat benützt, der aber folgendermassen abgeändert war: Die Geruchsgefässe wurden an den Enden der je 30 cm langen und 2 cm dicken Schenkelrohre des Y-Rohres angebracht, wie an dem von Schantz (1953) konstruierten Olfaktometer, das Absaugen der Luft geschah aber nicht an der Gabelungsstelle, sondern in der Mitte des 17 cm langen Einsatzrohres, sodass die Käfer schon vor dem Erreichen der Gabelungsstelle unbedingt mit beiden Gerüchen in Kontakt gekommen sind, was bei der üblichen Anordnung des Absaugrohres nicht gesichert ist. Die Geschwindigkeit des Luftstromes war in den Schenkelrohren etwa 8 m/min.

Die Käfer wurden in Gruppen von je 5 Exemplaren in den Apparat eingesetzt und nur jene Tiere ausgewertet, die bis zum Ende der Schenkelrohre liefen. Nach jeder Käfergruppe wurde der Apparat mit Hilfe eines Wattebausches, der auf ein Stück Draht befestigt und mit 96%igem Äthylalkohol durchtränkt war, ausgewaschen, nachher mit trockenen Wattebäuschen mehrmals gründlich durchgewischt, danach 10 Minuten lang gelüftet und die beiden Geruchsgefässe umgetauscht mit den Schenkelrohren wieder verbunden.

„Sandwich"-Test. Die Verzehrbarkeit der Blattscheiben verschiedener Pflanzen wurde mit der nach Langenbuch (1952) entwickelten „Sandwich"-Methode getestet. Aus Kartoffelblättern und aus den Blättern der zu untersuchenden Pflanze wurden mit Hilfe eines Korkbohrers Scheiben (Durchmesser 16 mm) ausgestanzt. Diese wurden in zwei Varianten mit Kartoffelstärke-Kleister zusammengeklebt, und zwar: A = zwischen zwei Kartoffelblattscheiben eine Scheibe der zu untersuchenden Pflanze, B = drei Kartoffelblattscheiben. Je ein „Sandwich" wurde zwischen zwei etwa 2 × 2 mm grossen und 1 mm dicken Korkplättchen auf einer Stecknadel befestigt. Petri-Schalen (Durchmesser 12 cm) wurden mit runden Wellpappenscheiben ausgestattet und letztere mit Filterpapier bedeckt. Die Stecknadeln mit den „A"-bzw. „B-Sandwiches" wurden abwechselnd in einem Kreis von 7 cm Durchmesser eingesteckt, sodass in jedes Gefäss 6 „A"- und 6 „B-Sandwiches" kamen. Die „Sandwiches" waren an den Stecknadeln so hoch befestigt, dass sie etwa 5 mm über dem Filterpapier standen und auf diese Weise von den Käfern rittlings verzehrt werden konnten. Um das schnelle Austrocknen der Scheiben zu verhindern wurde die Wellpappe in den Petrischalen mit je 2 ccm Wasser befeuchtet und darüber ein Glaszylinder (Durchmesser 11 cm) gestülpt, der oben mit einer zweiten Petri-Schale abgedeckt war (Abb. 1). Die Gefässe wurden von oben gleichmässig beleuchtet. In jedes Gefäss kamen 10—12 Stück 1—2 Tage alte Käfer, die bis Versuchsbeginn in mit nassen Wattebäuschen beschickten Petri-Schalen aufbewahrt wurden.

Zur Auswertung wurden in gewissen, von der Frassintensität abhängenden Zeitabschnitten (meist 1—2 Stunden) die von den mittleren Scheiben der „Sandwiches" verzehrte Fläche geschätzt und in einer Skala von 0 (unberührt) bis 1,00 (total verzehrt) ausgedrückt. Durch Kontrollmessungen konnte festgestellt werden, dass nach entsprechender Übung die Schätzung mit einem Fehler von nicht grösser als 0,05 durchgeführt werden kann. Da jeder Versuch in mindestens 4 Wiederholungen angelegt wurde und somit zur Schätzung mindestens 4 × 6 „A"- und ebensoviele „B-Sandwiches" zur Verfügung standen, ist der Fehler unwesentlich. Die Schätzung bedeutet gegenüber jeder Methode der direkten Flächenmessung eine ungeheure Ersparnis an Zeit und Arbeit. Zur endgültigen Auswertung wurden aus jeder Versuchsreihe, aus den nacheinander durchgeführten Ablesungen die Werte jener Ablesungen gewählt, bei denen die Kontrollscheiben (mittlere Scheiben der „B-Sandwiches") bis auf 50—80% verzehrt wurden.

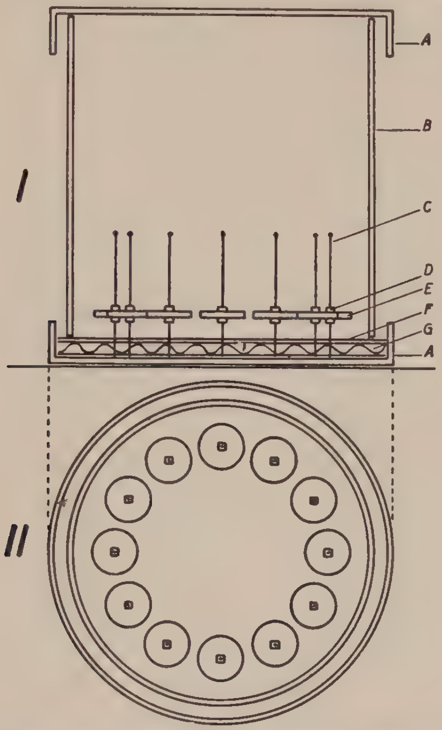

Abb. 1. Versuchsgefäss für den "Sandwich"- und Scheiben-Test. A = Petri-Schalen, B = Glaszylinder, C = Stecknadel, D = Korkplättchen, E = "Sandwich" oder Blattscheibe, F = Filterpapier, G = Wellpappe. I = Seitenansicht, II = Aufsicht.

Blattscheiben-Test. Zur Prüfung der frasshemmenden Wirkung verschiedener Stoffe wurden die ausgestanzten Kartoffelblattscheiben mit der zu untersuchenden Flüssigkeit im Vakuum infiltriert oder mit Hilfe einer Mikropipette an beiden Seiten mit einer bestimmten Flüssigkeitsmenge gleichmässig betupft oder in die Flüssigkeit getaucht und die Menge der aufgenommenen Flüssigkeit gemessen. Nach Antrocknen der Flüssigkeit wurden die Scheiben wie beim „Sandwich"-Test einzeln an Stecknadeln befestigt und diese in oben angegebener Weise in die Versuchsgefässe eingesteckt. In jedem Gefäss standen auch hier abwechselnd 6 mit der zu untersuchenden Flüssigkeit und 6 mit destilliertem Wasser behandelte Scheiben. Auch die Auswertung geschah wie beim „Sandwich"-Test.

Bei der Untersuchung der Orientierung der Käfer im Freiland wurde die Windrichtung in einer Höhe von 3—5 cm über der Bodenoberfläche mit kleinen, sich leicht drehenden Fähnchen laufend kontrolliert.

VERSUCHSERGEBNISSE

Das Verhalten der überwinterten Käfer beim Aufsuchen der Nährpflanze. Die während fünf Jahren (1953—1957) hindurch angestellten Freilandbeobachtungen zeigten, dass die überwiegende Mehrzahl der überwinterten Käfer nach dem Verlassen des Winterquartiers nicht sofort eine Affinität zur Wirtspflanze aufweisen, auch wenn ihnen Wasser in genügender Menge in Form von Tau und Regen zur Verfügung steht. Hauptsächlich die früher an der Bodenoberfläche erschienenen Käfer wandern bzw. fliegen eine Zeit lang umher, ohne auf die Anwesenheit der Wirtspflanze im geringsten zu reagieren. Werden solche Käfer an frisch aufgelaufene Kartoffelpflanzen gesetzt, so verlassen sie diese sehr oft und wandern oder fliegen weiter. Es ist demnach anzunehmen, dass die Diapause nicht in allen Beziehungen auf einen Schlag, sondern stufenweise aufhört, wobei erst die negative Geotaxis bzw. die positive Phototaxis, nachher die Wanderlust zum Ausdruck kommt, während sich das Bedürfnis der Nahrungsaufnahme erst später meldet (Dies könnte als eine Anpassungsreaktion der Art betrachtet werden, da die Solanaceen wärmebedürftige Pflanzen sind und im Frühling spät erscheinen).

Eine intensive Frasstätigkeit der überwinterten Käfer wurde in den oben angegebenen Jahren am Versuchsort (Keszthely, Westungarn) in der Regel erst nach Anfang Mai beobachtet, obzwar der Frühling in diesen Jahren beträchtlich verschieden war und die ersten Käfer bereits Ende März oder Anfang April an der Bodenoberfläche erschienen. Dieser Umstand erklärt auch die Tatsache, dass die mit vorgekeimten Kartoffelknollen angelegten Fangstreifen in der Praxis erfolglos waren, da mit ihrer Hilfe nur ein Bruchteil der überwinterten Käfer an den vorigjährigen Herden festzuhalten bzw. zu vernichten war (JERMY & SARINGER, 1955).

Aus obigen Beobachtungen folgt, dass bei den mit überwinterten Käfern durchgeführten Nahrungswahlversuchen die Unsicherheit des Bedürfnisses der Nahrungsaufnahme immer in Betracht gezogen werden muss.

Bei der Klärung der Frage, wie die überwinterten Imagines die Nährpflanze finden, liegt zunächst die Annahme auf der Hand, dass dabei die *olfaktorischen* Reize eine wichtige Rolle spielen könnten. McINDOO (1926) und neuerdings SCHANTZ (1953) hatten mit geeigneten Olfaktometer-Versuchen den Geruchssinn der Kartoffelkäfer-Imagines eindeutig bewiesen. GRISON (1956) stellte fest, dass der Geruch eines abgeschnittenen Kartoffelblattes von den Käfern im Labor schon aus einer Entfernung von 4 cm wahrgenommen wird. Auf Grund unserer Olfaktometer-Versuche müssen wir jedoch annehmen, dass die von den Käfern wahrnehmbare Geruchsintensität verhältnismässig hoch liegen muss. Wurden nähmlich die Geruchsgefässe des Olfaktometers mit ganzen Kartoffeltrieben beschickt (zum Vergleich dienten Luzernetriebe), so ergab sich — im Gegensatz zu den Ergebnissen von McIndoo — keine statistisch gesicherte Differenz bei der Wahl der Rohre. Wurden aber die Blätter derselben Pflanzen zerkleinert in die Geruchsgefässe hineingelegt, so wählten die Käfer sehr gut gesichert den Kartoffelduft. (Von 205 Tieren wählten 135, also 64,6% den Kartoffelduft; der χ^2-Test ergibt einen P-Wert von kleiner als 0,1). Eine so grosse Intensität des Geruches ist aber in der Natur wahrscheinlich nur in der nächsten Nähe der Kartoffelstauden vorhanden, sodass eine geruchliche Wahrnehmung der Wirtspflanze nur aus einer verhältnismässig kleinen Entfernung möglich zu sein scheint.

Diese Annahme wurde durch unsere Freilandsbeobachtungen bestätigt. Vom Verhalten der hungrigen Käfer in der Umgebung einer Kartoffelparzelle konnte eindeutig der Schluss gezogen werden, dass die olfaktorische Wahrnehmung der Pflanze höchstens aus einer Entfernung von nur wenigen Dezimetern möglich ist. Es zeigte sich aber, dass die Käfer bei der Nahrungssuche sehr lebhaft auf die von naheliegenden Gegenständen stammenden *visuellen* Reize reagieren. So laufen sie zu jeder Pflanze hin, die in einer Entfernung von 1—2 Dezimetern in ihrem Sichtfeld erscheint, klettern an den Pflanzen hoch und betasten diese sorgfältig mit den Palpen, auch wenn es sich nicht um eigentliche Wirtspflanzen handelt. Das Verhalten der Käfer ändert sich auch dann nicht, wenn in einer Entfernung von wenigen Dezimetern Kartoffelpflanzen vorhanden sind und die bodennahe Luftströmung den Geruch der letzteren den Käfern zubringt.

Die Olfaktostimuli scheinen also beim Auffinden der Wirtspflanze durch die überwinterten Käfer eine ganz unbedeutende Rolle zu spielen. Eine Unterscheidung der Pflanzen aus einer grösseren Entfernung ist für die Käfer unmöglich. Das Auffinden der Wirtspflanze geschieht demnach ganz zufällig. Die Olfaktostimuli bewirken wahrscheinlich nur das Festhalten der sich ernährenden und der eierlegenden Käfer an der Kartoffelstaude bzw. in deren nächster Umgebung, denn bei der Wahl der Eiablagestelle hat die Kontaktchemorezeption allem Anschein nach keine Bedeutung. Dadurch ist es zu erklären, dass man in der engen Umgebung der Kartoffelstauden — also vermutlich im Geruchsfelde dieser — oft auch an den Blättern anderer, als Wirtspflanze nicht in Betracht kommender Pflanzen Eigelege finden kann.

Das Verhalten der Jungkäfer beim Aufsuchen der Nährpflanze. Die vor 1—2 Tagen geschlüpften und bis Versuchsbeginn nur mit Wasser versorgten Jungkäfer wählten im Olfaktometer in 57,1% der Läufe (von 231 Individuen 132) den Luzerneduft ! Die Differenz kann nicht als gesichert betrachtet werden, da sich im χ^2-Test ein P-Wert von 2,45 ergibt. Wurden aber dieselben Käfer 24 Stunden lang mit Kartoffelblättern gefüttert und nach 24 stündigem Hungern im Olfaktometer geprüft, so wählten von 251 Individuen 155, also 61,8% den Kartoffelduft. Die Differenz ist sehr gut gesichert: der χ^2-Test gibt einen P-Wert von kleiner als 0,10. Es scheint also, dass der Jungkäfer nur nach erfolgter „Dressur" (d.h. nach Nahrungsaufnahme) auf die von der Wirtspflanze stammenden Olfaktostimuli anspricht (Vgl. die Versuche von GOTTSCHALK (1957) mit Geraniol beim Kartoffelkäfer).

Auch die mit noch nicht gefütterten Jungkäfern durchgeführten Freilandsversuche zeigten, dass die Tiere nicht von olfaktorischen Reizen zur Wirtspflanze geleitet werden. Es ist aber sehr auffallend, wie lebhaft auch die Jungkäfer auf *visuelle* Reize ansprechen. Falls im Laufe ihrer Wanderung bei der Nahrungssuche in ihrem Sichtfeld irgendwelche erhabene Gegenstände erscheinen, so laufen sie sofort hin. Wenn es sich um eine Pflanze handelt, so klettern sie meistens hinauf, betasten diese mit den Palpen sorgfältig und beissen die Blattränder oft an, auch wenn es sich nicht um eine Nährpflanze handelt.

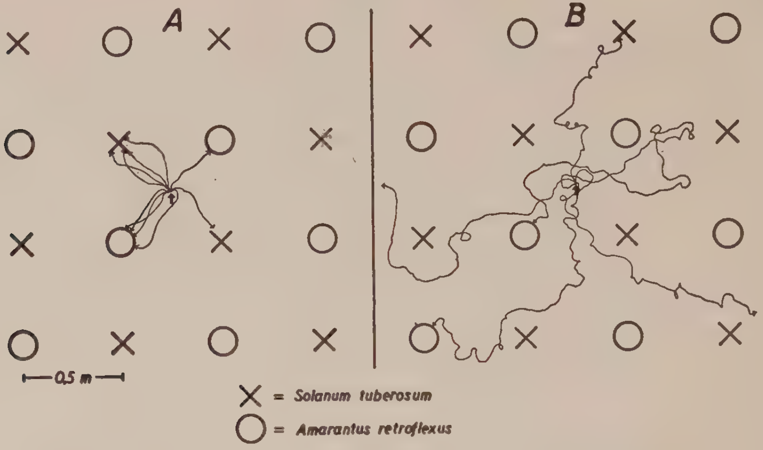

Abb. 2. Lauflinien normaler (A) und geblendeter (B) Jungkäfer zwischen etwa 15 cm hohen Kartoffel- und Amarantus-Pflanzen. Sonnenschein von links.

Abb. 2 zeigt einige Lauflinien normaler (A) bzw. mit weisser Ölfarbe geblendeter (B) Jungkäfer im Freiland zwischen ungefähr gleichgrossen, etwa 15 cm hohen Kartoffel- und Amarantus-Pflanzen. Der Verlauf der Linien deutet ganz klar darauf hin, dass die Jungkäfer durch visuelle Reize an die Pflanzen geleitet werden und dass sie die Nährpflanze von anderen Pflanzen nicht einmal aus einer Entfernung von 2 Dezimetern unterscheiden können. Die geblendeten Käfer können die Anwesenheit der Wirtspflanze auch in deren nächster Nähe nicht wahrnehmen, sie zeigen aber eine stark ausgeprägte negative Geotaxis, indem sie auf alle erhabenen Gegenstände, denen sie begegnen, wahllos hinaufklettern.

Im Laufe der an nahrungssuchenden Jungkäfern im Freiland durchgeführten Beobachtungen konnten wir noch feststellen, dass diese binnen einiger Minuten nach dem „Start'' eine mehr oder weniger *konstante Laufrichtung* aufnehmen und diese lange einhalten. Wie aus Abb. 3 ersichtlich, wird diese Richtung bei den verschiedenen Individuen weder durch die Windrichtung, noch durch die Richtung der Sonnenstrahlen bedingt. Werden die Käfer von der einmal eingeschlagenen Laufrichtung gewaltsam abgelenkt, so nehmen sie dieselbe nach einige Zentimeter langem Lauf meistens genau wieder auf. Diese Konstanz der Laufrichtung konnte nicht nur bei den auf einer kahlen Fläche, sondern auch bei den auf einer mit niedrigen Pflanzen spärlich bedeckten Fläche (zwischen jungen Unkräutern) wandernden Käfer festgestellt werden, trotzdem im letzteren Falle die Tiere von ihrer eigentlichen Laufrichtung durch die im Sichtfeld erscheinenden Pflanzen immer wieder abgelenkt werden (s. Abb. 3, Parzelle B). Das Einhalten einer konstanten Laufrichtung kann als eine Anpassungsreaktion betrachtet werden, die es ermöglicht, dass das eines entsprechenden Geruchssinnes entbehrende Tier binnen einer verhältnismässig kurzen Zeit verschiedene Pflanzenbestände durchquert, womit die Wahrscheinlichkeit des Auffindens einer geeigneten Wirtspflanze erhöht wird.

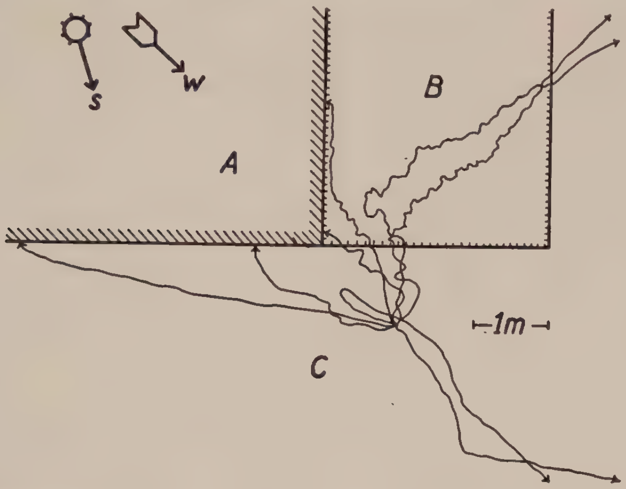

Abb. 3. Laufiinien einiger Jungkäfer in der Umgebung einer blühenden Kartoffelparzelle. A = Kartoffelparzelle, B = mit jungen Unkräutern spärlich bewachsene Fläche, C = kahle Fläche, S = Richtung der Sonnenstrahlen, W = Windrichtung.

Mit dieser konstanten Laufrichtung ist die in der Praxis oft gemachte Beobachtung zu erklären, dass beim Massenauftreten der Jungkäfer, nach dem Verdorren der Blätter der Frühkartoffeln, die vom Befallsherd abwandernden Käfer in einer gewissen Entfernung vom Herd — auf einer kleineren Fläche betrachtet — alle miteinander parallel wandern.

Die reflektorischen Bedingungen der Nahrungsspezialisation des Kartoffelkäfers. Aus Obigem folgt, dass das Entstehen und die Aufrechterhaltung des Teilkonnexes Kartoffelkäfer-Wirtspflanze weitgehend durch den *Geschmackssinn* der Käfer bedingt ist. Eine Störung des Teilkonnexes ist demnach durch die Beeinflussung der Geschmacksrezeption zu erwarten. In diesem Zusammenhange ist es sehr wichtig zu klären, ob die Nahrungsspezialisation (nur die Verzehrbarkeit, nicht aber den Ernährungswert der Pflanze berücksichtigend) mehr in einer Anpassung an spezifische, in den eigentlichen Wirtspflanzen vorhandene Frass-Stoffe oder aber in einer negativen Anpassung gegenüber vergällend wirkenden Stoffen zu suchen ist. Obwohl sich in den letzten drei Jahrzehnten mehrere Forscher mit den die Nahrungswahl des Kartoffelkäfers bedingenden Reizstoffen befassten, wurde diese Frage nicht eindeutig beantwortet.

Nach einigen Vorversuchen von RAUCOURT & TROUVELOT (1933, 1936) konnte CHAUVIN (1952) aus Kartoffelblättern einen Frass-Stoff isolieren, der wahrscheinlich ein Flavonderivat ist. HESSE & MEIER (1950) hatten bewiesen, dass Acetaldehyd schon in ganz geringen Mengen eine anlockende Wirkung auf den Kartoffelkäfer ausübt. THORSTEINSON (1955) meint, dass Acetaldehyd eher als ein Lockstoff bei der Auffindung der Nahrung (food finding stimulus) dient und nicht als Frass-Stoff zu betrachten ist.

Auf der anderen Seite wurden im Zusammenhang mit der Züchtung kartoffelkäferresistenter Kartoffelsorten die Stoffe eingehend untersucht, die für die Resistenz verantwortlich sind (vgl. die zusammenfassende Arbeit von SCHREIBER, 1957). Diese Arbeiten zeigten, dass dabei die Alkaloide eine ausschlaggebende Rolle spielen, und zwar weniger als Giftstoffe — wie es von manchen Autoren (CHIN, 1950; TROUVELOT & BUSNEL, 1937; SCHAPER, 1953) angenommen wird — sondern hauptsächlich als Vergällungsstoffe (LANGENBUCH, 1952; JERMY & SARINGER, 1955; BUHR, 1956; SCHREIBER, 1957).

Eigene Freilandbeobachtungen zeigten, dass die Jungkäfer bei der Nahrungssuche oft die Blätter der verschiedensten Pflanzen, und zwar Gramineen, *Veronica-, Chenopodium*-Arten, *Amarantus retroflexus, Convolvulus arvensis, Lepidium draba, Glechoma hederacea* anbeissen können, ohne dass eine kontinuierliche Frasstätigkeit an diesen Pflanzen zu beobachten wäre. In Nordamerika hat man den gelegentlichen Frass der Käfer an folgenden Pflanzen beobachtet: *Cirsium, Chenopodium*, Hafer, Kohl, *Amarantus, Sisymbrium, Polygonum, Eupatorium, Verbascum, Rubus* (TROUVELOT, 1936). Von den Blättern der im Schatten gewachsenen jungen *Galinsoga parviflora* Pflanzen können die Jungkäfer sogar beträchtliche Stücke verzehren (s. weiter unten).

TABELLE I

Verzehrbarkeit der Blätter verschiedener Pflanzen im „Sandwich-Test". (5 Wiederholungen; August, 1957)

Pflanze	Verzehrbarkeit (%)
Amarantus retroflexus	Spuren
Chenopodium album	20,0 ± 5,9
Phaseolus vulgaris var. *nanus*	26,1 ± 6,1
Brassica oleracea var. *cauliflora*	47,1 ± 14,4
Sonchus oleraceus	54,7 ± 9,8
Galinsoga parviflora (von schattigem Standort)	78,8 ± 8,2
Solanum tuberosum	100

Mit Hilfe des „Sandwich"-Testes konnte eindeutig festgestellt werden, dass die Befallsresistenz aller von uns untersuchten, nicht zu den Solanaceen gehörenden Pflanzen auf eine *vergällende* Wirkung zurückzuführen ist. Die Intensität dieser Wirkung zeigt eine ausserordentliche Mannigfaltigkeit von einer absoluten Befallsfreiheit bis zum gelegentlichen Befall. So erhielten wir z.B. in einer Versuchsreihe die in Tabelle I angegebene Werte für die Verzehrbarkeit der Blätter verschiedener

Pflanzen (Die von der Blattscheibe der fremden Pflanze verzehrte Fläche ist in Prozenten der von der mittleren Scheibe der „B-Sandwiches" verzehrten Fläche ausgedrückt).

Die Tatsache, dass die Käfer die Blätter der verschiedensten Pflanzen anbeissen und dass die kontinuierliche Frasstätigkeit an diesen Pflanzen durch die Anwesenheit vergällender Faktoren gehindert wird, zeugt davon, dass die Frass-Stoffe sehr verbreitet vorkommen, also nur begrenzt spezifisch sind (vgl. THORSTEINSON, 1958). Die Ursache der Nahrungsspezialisation des Kartoffelkäfers ist also weniger in einer Anpassung an spezifische, in den eigentlichen Nährpflanzen vorhandene Frass-Stoffe, als vielmehr in einer engen, *negativen Anpassung an vergällend wirkende Faktoren* (Vergällungsstoffe, eventuell pH, Turgor, mechanische Eigenschaften des Blattes, usw.) zu suchen. Demnach hat WARDLE's (1929) Behauptung, dass bei den Insekten eher von einer „Wirtsmeidung" (host avoidance) als von einer „Wirtswahl" (host selection) gesprochen werden kann, im Falle des Kartoffelkäfer volle Gültigkeit. Daraus folgt aber, dass der Frassreflex auch an den einen optimalen Frass-Stoffgehalt aufweisenden, eigentlichen Wirtspflanzen mit verschiedenen Stoffen gehemmt werden kann.

So ist die repellente Wirkung der Kupferverbindungen allgemein bekannt (vgl. DETHIER, 1947), ohne dass man dieser Tatsache besondere Aufmerksamkeit gewidmet hätte. Um die vergällende Wirkung der Bordeaux-Brühe näher zu bestimmen, wurden aus den mit einer 0,75%-igen Bordeaux-Brühe behandelten Kartoffelblättern Scheiben gestanzt und im Scheiben-Test untersucht. Es ergab sich eine Verzehrbarkeit von nur 59,3 ± 5,3% gegenüber 100% der mit Wasser behandelten Kartoffelblattscheiben. Wurden die Blätter an beiden Seiten mit einer 1%-igen Brühe gründlich behandelt, so sank die Verzehrbarkeit noch wesentlicher (s. Tabelle II).

Die abschreckende Wirkung des Tetramethyl-thiuram-disulphids wurde bereits von GUY (1936, zit. nach DETHIER, 1947) festgestellt und konnte durch unsere Versuche ebenfalls bestätigt werden. Im Scheiben-Test konnten wir eine praktisch totale Frasshemmung mit verschiedenen Alkaloiden (1%-ige Lösungen von Arbutin, Brucin, Veratrin, Spartein in die Scheiben infiltriert) erreichen. Die 1%igen Lösungen von Natriumtartarat und Natriumformiat zeigten in einer Menge von 0,5 ccm pro 100 qcm Blattoberfläche eine erhebliche Frasshemmung. Die vergällende Wirkung von vier Fungiziden ist in Tabelle II zusammengestellt.

TABELLE II

Vergällende Wirkung fungizider Spritzbrühen gegen Jungkäfer (Scheiben-Test, 5 Wiederholungen, 0,5 ccm Brühe pro 100 qcm Blattoberfläche).

Mittel	Konz. %	Verzehrbarkeit %
Bordeaux-Brühe	1	9,4 ± 1,7
Dinitro-rhodanbenzol	0,5	21,0 ± 6,9
Eisen-dimethyl-dithio-carbamat	0,5	32,4 ± 5,2
Natrium-dimethyl-dithio-carbamat	0,5	44,6 ± 11,9
Kontrolle (Wasser)	—	100

Betrachtet man neben den von SCHREIBER (1957) gründlich untersuchten Solanum-Alkaloiden auch die oben angeführten, vergällend wirkenden Stoffe, so tritt es klar zu Tage, dass die Vergällung auch beim Kartoffelkäfer nicht an eine bestimmte chemische Struktur gebunden ist, wie das bereits CHAUVIN & MENTZER (1951) für die gegen Acrididen wirksamen Vergällungsstoffe festgestellt haben. Diese Tatsachen bestätigen DETHIER's (zit. nach SCHREIBER, 1957) Auffassung, die nämlich, dass die Chemorezeption weniger als ein biochemischer, sondern eher als ein biophysikalischer Prozess anzusprechen ist. Demnach ist anzunehmen, dass entsprechend wirksame Vergällungsstoffe unter den verschiedensten Verbindungen anzutreffen wären, die zweckmässigerweise auch andere vorteilhafte Eigenschaften besitzen (z.B. Fungizide oder Blattdünger).

Um festzustellen, wie sich die Vergällung der Nahrung auf die Weiterentwicklung der Jungkäfer auswirkt, wurden im August 1957 im Gewächshaus in 6 Wiederholungen je 24 Jungkäfer (12 ♂, 12 ♀) mit von 1%-iger Bordeaux-Brühe bzw. mit Wasser gründlich bespritzten Kartoffeltrieben gefüttert (tägliche Beleuchtung 20 Stunden). Die Ergebnisse sind in Tabelle III zusammengefasst.

TABELLE III

Einfluss der Vergällung der Nahrung auf die Weiterentwicklung der frischgeschlüpften Jungkäfer (6 Wiederholungen).

Behandlung der Kartoffeltriebe	Benehmen der Käfer	Mortalität nach 21 Tagen	Eier je Weibchen nach 21 Tagen
1%-ige Bordeaux-Brühe	Vereinzelt Randfrass an den Blättern. Stengel befressen. Käfer wandern herum.	63%	0
Wasser	Normaler Frass an den Blättern. Stengel unbeschädigt. Käfer ruhig.	4%	33,5

Die Versuchsergebnisse zeigen, dass schon eine mit der Bordeaux-Brühe erreichte Vergällung einen tiefgreifenden Einfluss auf die Populationsdynamik des Schädlings ausüben kann.

SCHLUSSFOLGERUNGEN

Die enge Anpassung des Kartoffelkäfers gegenüber vergällenden Stoffen gibt uns zwei Möglichkeiten zur Ausarbeitung selektiver Bekämpfungsmethoden, mit deren Hilfe eine gewisse Herabsetzung des Vermehrungspotentials und damit wenigstens der teilweise Ersatz der insektiziden Behandlungen möglich zu sein scheint.

Die erste Möglichkeit bietet sich in einer entsprechenden *Veränderung des Reizstoffgehaltes der Wirtspflanze.* Seit mehr als zwei Jahrzehnten werden grosse Anstrengungen zur Herstellung larvenresistenter Kartoffelsorten gemacht, ohne jedoch entsprechende Resultate erreichen zu können (BUHR, 1956). Es wäre die Frage zu prüfen, ob nicht schon durch eine weniger tiefgreifende Veränderung des Reizstoffgehaltes, die Frassintensität der Käfer genügend herabgesetzt werden könnte, um dadurch die Reproduktionsrate bis auf ein wirtschaftlich ertragbares Niveau zu senken. — Andererseits wäre auch zu untersuchen, ob nicht auch durch gewisse Kulturmassnahmen derartige Veränderungen in der Pflanze hervorgerufen werden könnten. Bekanntlich wurde in mehreren Fällen bewiesen, dass die Bodenzusammensetzung bzw. die Anwendung gewisser Düngemittel den Befall der Pflanzen durch verschiedene Schädlinge erheblich beeinflussen kann (ARENZ & SCHRÖPPEL, 1952; MERKER, 1955; BÜTTNER, 1956) und es ist anzunehmen, dass die erhöhte Mortalität der Schädlinge auch in diesen Fällen wenigstens teilweise durch eine Veränderung des Reizstoffgehaltes der Pflanzen bedingt ist. Es sei hier noch auf die Beobachtungen von CHAUVIN (1951) hingewiesen, der feststellen konnte, dass der Frass-Stoffgehalt der Kartoffelblätter auch in Abhängigkeit vom Standort beträchtlich variieren kann.

Die zweite Möglichkeit liegt in der *Anwendung von Vergällungsstoffen.* Die mit der Bordeaux-Brühe gemachten Erfahrungen zeugen davon, dass in dieser Hinsicht vielversprechende Ergebnisse zu erwarten sind, falls man noch stärker wirkende Stoffe entwickeln könnte. In diesem Zusammenhange seien beispielsweise die Untersuchungen von SMITH (1957) erwähnt, der feststellen konnte, dass die in der praktischen Bekämpfung von *Conotrachelus nenuphar* bewährten Mittel (Bleiarsenat, Netzschwefel, Kalkmilch) den Befall nicht durch insektizide, sondern durch vergällende Wirkung auf einen erträglich niedrigen Wert zurückdrängen. Die Anwendung von Repellents an Stelle rasch wirkender Insektizide mag zunächst befremdlich sein, denn man hat sich zu sehr an die „guten" Schädlingsbekämpfungsmittel gewöhnt. Die einseitige Einstellung auf totale, schnell und andauernd wirkende Gifte sollte jedoch aus human-, tier- und biozönosehygienischen Gründen dringend einer weiterblickenden Anschauung Platz machen.

SUMMARY

In order to find selective control methods, which would not disturb the biocoenoses, the reflex mechanism of food finding and host selection by the Colorado potato beetle was investigated. The greater part of the overwintered beetles do not begin to feed just after emergence, but first migrate. Seeking the food they perceive the host plant in the field by olfaction from a distance of not more than a few decimetres. The newly hatched young beetles react upon the olfactostimuli coming from the host plant only after some "training" (= feeding). During the food-seeking of old and young beetles sight only plays an important role, however, within a very limited distance. If searching for food, young beetles keep a constant direction in creeping. In the Colorado beetle host selection depends mainly on taste. Feeding stimuli are not markedly specific; the oligophagy of the beetles is based largely upon the negative reactions to repellent stimuli. Repellency is to a great extent independent of chemical structure and is therefore to be found in the many different groups of compounds. For example the repellency of the Bordeaux mixture is very high and is thereupon able to reduce the rate of reproduction very considerably. The selective reduction of the biotic potential of the pest could be achieved (1) by changing the quantity of acceptants and rejectants in the host plant (by selection or by cultural methods) or (2) by applying repellents.

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DIE WIRKUNG VON EINIGEN PFLANZLICHEN SONDERSTOFFEN, INSBESONDERE VON ALKALOIDEN, AUF DIE ENTWICKLUNG DER LARVEN DES KARTOFFELKÄFERS (*LEPTINOTARSA DECEMLINEATA* SAY)

VON

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Die Wirkung von 83 Alkaloiden, Saponinen und einigen weiteren Naturstoffen bzw. ihren Derivaten wurde bei Zwangsfütterungen auf die Larven des Kartoffelkäfers getestet. Die ermittelten Befunde werden tabellarisch wiedergegeben und kurz diskutiert.

Die Nahrungs- und die Brutpflanzen des Kartoffelkäfers gehören ausnahmslos zu der Familie der Solanaceen, einer Pflanzenfamilie, die in chemischer Hinsicht durch ihren Reichtum an Alkaloiden ausgezeichnet ist. Jedoch stellen nicht alle Solanaceen eine für dieses Tier geeignete Nahrung dar. Einige von ihnen werden, wie auch andere Pflanzen, zwar vom Käfer ganz ohne Zusammenhang mit der Nahrungssuche rein zufällig besucht, aber nicht von ihm „befallen" oder mit Eiern belegt. Doch können legereife Weibchen in Zwangslagen sogar unter Freilandverhältnissen ihre Eier an Pflanzen verschiedenster Artzugehörigkeit und selbst an totem Material in zuweilen sogar normalen Gelegen absetzen, worauf allerdings die zu grösseren Ortsveränderungen nicht befähigten Eilarven in der Regel zugrunde gehen.

Das Ausbleiben des Befalls kann bei den Pflanzen durch verschiedene Umstände bedingt sein, sei es, dass sie die befallsnotwendigen Lockstoffe („*attractants*") nicht enthalten, sei es, dass sie mit befallsabweisenden Stoffen ("*repellents*") ausgestattet sind. Die chemische Charakterisierung derartiger, für das Verhalten des Kartoffelkäfers ausschlaggebender Stoffe steht noch aus. Verwandschaftliche Beziehungen bzw. die artspezifischen Eiweißsubstanzen der Pflanzen spielen im einzelnen, ähnlich wie bei der Wirtswahl einer Anzahl von weiteren oligophagen Pflanzenparasiten (vgl. BUHR, 1937), so auch für die Substratwahl des Kartoffelkäfers, keine generell ausschlaggebende Rolle.

Auch nach vollzogenem Befall ermöglichen keineswegs alle Solanaceen eine ungestörte Entwicklung des Kartoffelkäfers oder seiner Larven. Einerseits können den Substraten die zum Befressenwerden notwendigen Fraßstoffe („*phagostimulants*") fehlen, andererseits können die in den betreffenden Pflanzen vorhandenen Nähr- oder Wirkstoffe für einen vollen Lebensablauf nicht ausreichen. Weitere Pflanzen besitzen Inhaltsstoffe, welche je nach Konzentration auf die Tiere vergällend oder sogar abschreckend („*rejectant*") wirken, und manche Solanaceen schliesslich weisen Inhaltsstoffe auf, die, ohne dass sie in allen Fällen gleichzeitig eine vergällende Wirkung zeigen, in unterschiedlicher Weise auf das Tier toxisch wirken (vgl. auch DE WILDE, 1958).

Durch die Untersuchungen von KUHN und Mitarbeitern (u.a. 1947, 1950, 1957) wurde bekannt, dass einige von den knollentragenden *Solanum*-Arten sowie die Tomaten Stoffe enthalten, die in gewissen Konzentrationen eine futtervergällende Wirkung vor allem auf die Larven, in geringerem Maße auch auf die Adulten des Kartoffelkäfers ausüben. Weil diese Befunde für die Züchtung resistenter Kultursorten von besonderer Bedeutung sind, wurden von zahlreichen Autoren der verschiedensten, am Gesamtproblem interessierten Arbeitsrichtungen mannigfaltige Untersuchungen über die Alkaloide der *Solanum*- und der *Lycopersicon*-Arten durchgeführt (vgl. SCHREIBER, 1954, 1955b, 1957, 1958).

Auffallend ist, dass sich der Kartoffelkäfer an einigen Solanaceen, die aufgrund ihrer Inhaltsstoffe eine zum Teil starke Wirksamkeit auf den Wirbeltierorganismus ausüben, anscheinend normal zu entwickeln vermag (*Atropa belladonna* und Formen, manche *Hyoscyamus*-, *Datura*- und *Nicotiana*-Arten; vgl. BUHR, 1956, 1958). Weitere Arten aus den zuletzt genannten Gattungen erwiesen sich demgegenüber als mehr oder weniger resistent. Diese unterschiedlichen Befunde führten zu der Frage, ob auch in diesen Fällen Zusammenhänge zwischen der Käferwiderstandsfähigkeit und dem Alkaloidvorkommen in den betreffenden Substraten bestehen. Einzelergebnisse über die an der Forschungsstelle für Kartoffelkäfer-Bekämpfung Mühlhausen in dieser Hinsicht durchgeführten Untersuchungen wurden speziell im Hinblick auf die *Solanum*-Arten bereits in mehreren der bisher genannten Mitteilungen erwähnt. Mit den folgenden Ausführungen sollen die wichtigsten Ergebnisse, die überwiegend bei Zwangsfütterungen von Eilarven durch Testung von experimentell isolierten oder von im Handel bezogenen Solanaceen-Inhaltsstoffen bzw. auch von einigen ihrer Abkömmlinge (1—33)[1]) gewonnen wurden, im Zusammenhange dargestellt werden. Verschiedene Naturstoffe (34—83), insbesondere Pflanzenalkaloide und deren Derivate (34—66), wurden ebenfalls untersucht, um eventuell vorhandene Beziehungen zwischen einer etwaigen Käferwirksamkeit und der chemischen Konstitution dieser Substanzen zu ermitteln. Eine ausführlichere Besprechung dieser hier für Eilarven nur ausschnittsweise dargestellten Ergebnisse muss, ebenso wie entsprechende Untersuchungen mit zuvor normal ernährten L_2, L_3, L_4 oder Käfern, weiteren Mitteilungen, die auf spezielle Pflanzen- bzw. chemischen Stoffgruppen abgestellt werden sollen, vorbehalten bleiben. Auch die Befunde, welche bei histologischen und cytologischen Untersuchungen am Darmtractus von Tieren verschiedener Versuchsserien gemacht wurden, sollen in anderem Zusammenhange zur Darstellung kommen.

MATERIAL UND METHODE

Die verwendeten Larven stammten von Käfern, welche im Freilande gesammelt und erforderlichen Falles im Keller oder im Gewächshaus überwintert waren. Für jeden Versuch wurden zehn, auf Gleichmässigkeit ausgesuchte Larven benutzt, die ihren Eischalenfrass beendet hatten und sich anschickten, das Gelege zu verlassen. Als Futter dienten die Endfiedern von jungen, ausgewachsenen Blättern gesunder Stauden der Sorte „*Aquila*", und zwar von März bis Juni von Gewächshaus- bzw. Mistbeetpflanzen, von Juli bis September von Freilandpflanzen. Die getesteten Verbindungen wurden zum Teil aus entsprechendem Pflanzenmaterial in kristalliner oder in weitestgehend gereinigter Form isoliert, aus den Alkaloidglykosiden durch Abbau (Aglykone) bzw. partialsynthetisch (z.B. Demissidin aus Solanidin, vgl. ferner SCHREIBER, 1955a) gewonnen oder andernteils auf dem Handelswege bezogen.

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[1]) Vgl. Fussnote 1 auf S. 212.

Nach Möglichkeit wurden die zu prüfenden Substanzen bzw. ihre Salze in den gewünschten Konzentrationen unter Zusatz von 5% Gelatine in Wasser gelöst. Diese Testlösungen wurden nach Erwärmung im Wasserbad auf etwa 50° C in der von KUHN & GAUHE (1947) beschriebenen Weise mit Hilfe eines mehrfach abzurollenden Glasstabes so aufgetragen, dass — meist erst nach mehrfachem Bestreichen — eine Blattseite mit einem möglichst gleichmässigen Film überzogen war. Wägungen der Blattfiedern vor und nach dem Auftragen ermöglichten die Berechnung der auf der Fläche (mg Substanz/100 mg Blatt-Frischgewicht) vorhandenen Substanzmengen. Vor allem zur Darbietung in Wasser unlöslicher Stoffe wurde als Trägersubstanz Eiweißglycerin verwendet. Die weitgehend homogenen Verreibungen wurden mit gummigeschütztem Finger auf den Blättern verteilt. Das so präparierte Futter wurde nach entsprechenden Wägungen in gleicher Weise für die Versuche verwendet wie das mit Gelatinelösungen behandelte. Kontrollversuche ohne weitere Zusatzstoffe zeigen, dass auch Blätter mit einem Film von Eiweißglycerin ebenso wie die mit Gelatinelösung bestrichenen von den Larven ohne besonderes Zaudern gefressen werden und keine Entwicklungsstörungen bewirken.

Nachdem die aufgetragenen Filme hinreichend abgetrocknet waren, wurden die Blätter den Larven auf Dahlemer Hygrostatenschalen als Nahrung vorgesetzt, worauf das Verhalten und der weitere Entwicklungsablauf der Tiere, die in einer Temperaturkammer (23—24° C) gehalten wurden, täglich zur Bonitierung kam. In den ersten 5 bis 6 Tagen wurde das Futter nur alle 48 Stunden erneuert, anschliessend in 24stündigem Wechsel. Da zu jeder Versuchsserie mehrere Blätter benötigt werden, und da es nicht gelingt, die aufgetragenen Filme in allen Fällen einheitlich herzustellen, müssen die aufgetragenen Stoffmengen für jedes Blatt ermittelt werden. Die in der Tabelle I, Spalte 3, angeführten Konzentrationsangaben stellen die Mittelwerte aller in der gleichen Serie verwendeten Proben dar.

Die Entwicklung der Tiere wurde bis zum Absterben aller Larven oder bis zur Erlangung der Verpuppungsreife verfolgt. Zur Verringerung des ohnehin reichlich anfallenden Arbeitsaufwandes, teils auch zum Einsparen der oft schwierig zu beschaffenden oder kostbaren Testsubstanzen, wurde die Zahl der Versuchstiere bei Serien mit hinreichend verträglichem Futter im Laufe des Versuches in vielen Fällen auf etwa 5 Larven reduziert (vgl. Tabelle I, vorletzte Spalte). Bei den ausgeschalteten Tieren handelte es sich in allen Fällen um vitale Larven, die mit grosser Wahrscheinlichkeit ihre weitere Entwicklung ungestört beendet haben würden. Alle im Versuch verbliebenen Larven standen, auch wenn es sich dabei um Kümmerer oder Schwächlinge handelte, solange unter Beobachtung, bis ihre weitere Entwicklungsfähigkeit geklärt war.

VERSUCHERGEBNISSE

In der nachstehend, stark gekürzt wiedergegebenen Tabelle wurden die einzelnen Testserien gruppenweise zunächst nach pflanzenverwandtschaftlichen und dann innerhalb dieser Gruppen vorwiegend nach chemischen Gesichtspunkten angeordnet. Es folgen Angaben über Konzentration und Darbietungsweise der verabfolgten Stoffe, anschliessend Daten über den Entwicklungszustand der Larven an den hier nur auszugsweise wiedergegebenen Kontrolltagen, wodurch der Entwicklungsverlauf der Tiere im Vergleich zu den gleichzeitig durchgeführten Kontrollserien gekennzeichnet sein dürfte.

Während von den *Solanum-* bzw. *Lycopersicon-* Alkaloidglykosiden das α-Solanin, sein Aglykon Solanidin, die hieraus partialsynthetisch dargestellten Solanidinglykoside sowie die Roh-Alkaloide aus *Solanum tuberosum* und *Solanum chacoense* (1—9)[1]) die Entwicklung der Larven praktisch nicht beeinträchtigten, führten die Tetraoside[2]) vom Typ des Tomatin (12, 13, 17, 23, 24) sowie das Soladulcingemisch (22) zu erheblichen Entwicklungsbeeinträchtigungen. Eine intermediäre Wirkung konnte für Dihydro-α-solanin (10), Solacaulin (11), Polyadenin (16) und eventuell für das Roh-Solanigrin (21) festgestellt werden. Demissidinglucosid (15), Solasonin (18) und Solamargin (19) erwiesen sich, ähnlich wie das Solanin, als larvenunwirksam. Eigenartigerweise wurde bei der Testung der Aglykone Demissidin (14), Solasodin (20) und Tomatidin (24) eine gewisse Wirksamkeit festgestellt, die aufgrund der durch ihre Wasserunlöslichkeit schwierigen Applizierbarkeit nur mit Vorbehalt gewertet werden kann, eine Tatsache, die auch für das Solanidin (5) in Rechnung zu setzen ist.

Es mag darauf hingewiesen sein, dass bemerkenswerte Unterschiede zwischen der Wirksamkeit der freien Basen und ihren verschiedenen Salzen nicht festgestellt werden konnten. Dieses ergab sich bei zahlreichen, wegen ihrer Gleichartigkeit hier nicht aufgeführten Versuchsserien (z.B. 12 u. 13). Für die Larvenwirksamkeit dieser Alkaloidglykoside werden biophysikalische Vorgänge verantwortlich gemacht, die, ohne nachweisbare toxische Wirkung, die geschmackliche Vergällung der Nahrung verständlich machen. Volle Aktivität scheinen nur solche Alkaloide zu besitzen, bei denen bestimmte konstitutive Besonderheiten im Molekül gleichzeitig vorhanden sind (SCHREIBER, 1957, 1958).

Von den geprüften weiteren, nativ in den Pflanzen nicht in glykosidischer Bindung auftretenden Solanaceen-Alkaloiden besitzt das Nicotin bereits in geringen Konzentrationen ($>$ 0,0075 mg/100 mg Frischsubstanz (31—33)) eine auffallend starke Käferwirksamkeit, die im Gegensatz zu der offenbar vorwiegend vergällenden Wirkung der bisher erwähnten Glykoalkaloide eine ausgesprochen toxische ist. Pfropfungsversuche bestätigen diese Befunde. So erwiesen sich z.B. auf Kartoffelunterlagen weitestgehend nicotinarm aufgewachsene Reiser von normal voll resistenten *Nicotiana*-Arten als voll anfällig, und reziprok waren auf Tabakunterlagen entstehende Kartoffelreiser zufolge ihres nunmehr vorhandenen Nicotingehaltes weitgehend resistent (BUHR, 1954).

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[1]) Die in Klammern wiedergegebenen Zahlen verweisen auf die Nummern der betreffenden Substanzen in der Tabelle I.

[2]) Nach neueren, noch unveröffentlichten Untersuchungen von SCHREIBER enthalten auch die bei uns kultivierten, im Herbst geernteten Herkünfte von *Solanum demissum* in den Blättern zum mindesten als Hauptalkaloide ebenfalls Tomatidinglykoside.

Atropin und einige seiner Derivate (25—27, 29) zeigen in geringen Konzentrationen, wie sie im Blattmaterial normalerweise vorliegen dürften, keinen Einfluss auf die Larvenentwicklung. Erst höhere Konzentrationen von etwa 0,20 bis 1,50% im Frischgewicht bedingen bei den späteren Larvenstadien, besonders bei den einsetzenden Verpuppungsvorgängen, eine wesentlich erhöhte und mit zunehmender Konzentration ansteigende Mortalität, obwohl eine merkliche Störung der voraufgehenden Larvenentwicklung kaum oder nicht in Erscheinung tritt. Etwa

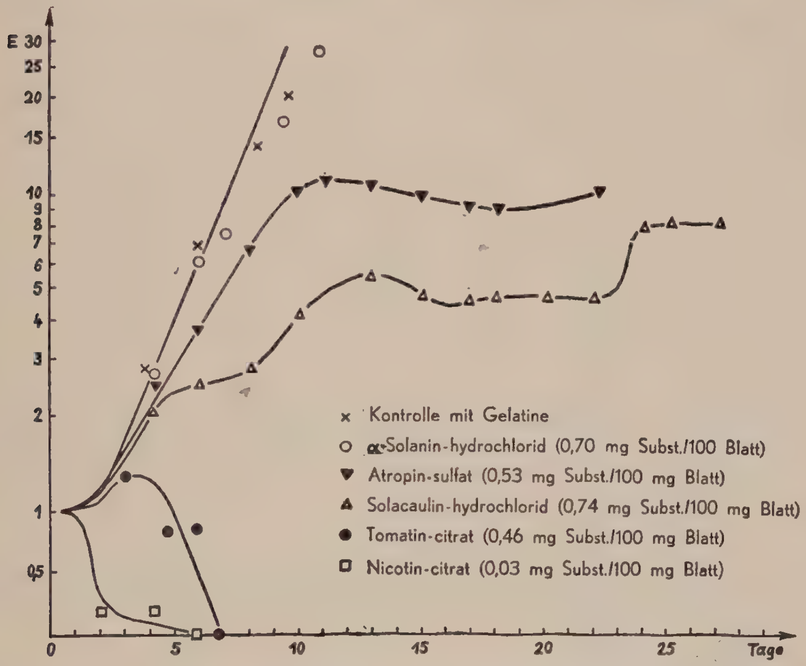

Abb. 1. Einfluss einiger Solanaceen-Alkaloide auf den Entwicklungsgang von Eilarven des Kartoffelkäfers (*Influence of some Solanaceae alkaloids on the course of development of larvae of the potato beetle)*

Abzisse: Zeit in Tagen. Ordinate: Entwicklung der Larven; die hier wiedergegebenen Zahlenwerte wurden an Hand der in der Tabelle I wiedergegebenen Daten nach folgender Gleichung errechnet:

(Abscissa: time in days. Ordinate: development of the larvae; the figures given here were calculated from the data given in Table I by the following equation:)

$$E_t = \frac{u_t \times 0 + v_t \times e^0 + w_t \times e^1 + x_t \times e^2 + y_t \times e^3 + z_t \times e^4}{u_t + v_t + w_t + x_t + y_t + z_t}$$

Darin bedeuten E_t = Entwicklungswert zur Zeit t; u_t = Anzahl der toten Larven zur Zeit t und entsprechend v_t, w_t, x_t, y_t, z_t die Anzahl der zur Zeit t vorhandenen L_1, L_2, L_3, L_4 bzw. der verpuppungsreifen Larven; e = 2,718.

(where E_t *= development value at time t;* u_t *= number of dead larvae at time t, and correspondingly* v_t, w_t, x_t, y_t, z_t *the number of* L_1, L_2, L_3, L_4 *and pupating-ripe larvae, present at time t; e = 2.718)*

TABELLE I

Übersicht über die durchgeführten Versuche und ihre gleichlaufenden Kontrollen

Die Zahlen in den Spalten 4—8 und zum Teil 10 bedeuten die an den betreffenden Tagen vorhanden L_1, L_2, L_3, L_4, P = zunächst verpuppungsreife Larven, später Puppen; (in Klammern) = Zahl der toten Tiere. In Spalte 3 bedeuten: G = Versuche, bei denen die Substanz in wässeriger Gelatine gelöst, E = solche, bei denen die Substanz in Eiweissglycerin verteilt verabfolgt wurde. KG bzw. KE kennzeichnen die parallel laufenden Kontrollserien, bei welchen nur die genannten Trägersubstanzen ohne Zusatz der zu prüfenden Stoffe geboten wurden. Die in dieser Spalte gleichzeitig angeführten Konzentrationsangaben beziehen sich auf mg Substanz pro 100 mg Blattfrischgewicht. Die in Spalte 2 zu rechts unten angegebenen Zahlen verweisen auf Monate und Jahr der Versuchsdurchführung.

Substanz			Stand der Larvenentwicklung an dem betreffenden Tage nach Versuchsbeginn					Bemerkungen	
Name (Vorkommen)	Konzentration im Test		2.	6.	10.	15.	20.	am Tage [n] wurden an Larven entfernt	am Tage [n] waren vorhanden
Normalentwicklung (t-Kammer, ca. 24°)	Kontrollen		8.2.0.0.	0.4.6.0.	0.0.2.7. (1)	0.0.0.1. 7P (2)	0.0.0.1. 7P (2)		
Normalentwicklung (Zimmertemperatur)			8.2.0.0.	0.5.4.0. (1)	0.0.4.4. (2)	0.0.0.4. 4P (2)	0.0.0.1. 7P (2)		
Hungertiere			10.0.0.0.	0.0.0.0. (10)					[5] 3L_1 (7)
1. *α*-Solanin-hydrochlorid (aus *S. tuberosum*) 6/55		KG	10.0.0.0.	0.0.10.0.	0.0.0.1. 3P (1)			[9] 5L_4	[12] 4P (1)
	0,23	G	10.0.0.0.	0.1.9.0.	0.0.1.9.	0.0.0.1. 5P		[10] 4L_4	[16] 6P
	0,70	G	10.0.0.0.	0.0.9.0. (1)	0.0.0.3. 1P (5)			[9] 5L_4	[14] 4P (1)
	2,29	G	9.1.0.0.	0.3.7.0.	0.0.0.5.	0.0.0.1. 4P	0.0.0.1. 4P	[9] 5L_4	[37] 4P (1)
2. Rohsolanin-citrat (Solanine u. Chaconine aus *S. tuberosum*) 6—7/55		KG	10.0.0.0.	0.3.7.0.	0.0.3.7.	0.0.0.1. 4P		[10] 5L_4	[16] 5P
	0,25	G	8.2.0.0.	0.0.9.0. (1)	0.0.0.4. (1)	0.0.0.1 3P (1)		[9] 5L_4	[16] 4P (1)
	0,92	G	7.3.0.0.	0.1.9.0.	0.0.0.4. (1)	0.0.0.1 3P (1)		[9] 5L_4	[18] 4P (1)
	1,09	G	10.0.0.0.	0.6.4.0.	0.1.6.2. (1)	0.0.1.3. (1)	0.0.0.2. 1P (2)	[13] 5L_4	[21] 2P (3)
3. Rohsolanin-citrat (Solanine u. Chaconine aus *S. chacoense*) 8/53		KG	10.0.0.0.	1.6.2.0. (1)	1.0.8.0. (1)		0.0.0.1. 5P (4)		[27] 6P (4)
	0,52	G	10.0.0.0.	0.10.0.0.	0.3.7.0.		0.0.0.5. 4P (1)		[26] 8P (2)
	1,47	G	10.0.0.0.	0,10.0.0.	0.4.6.0.		0.0.1.8. (1)		[41] 1L_4 4P (5)
4. Solanidin-acetat (aus *α*-Solanin) 9/53		KG	10.0.0.0.	1.9.0.0.	0.1.9.0.	0.0.1.8. (1)	0.0.0.1. 6P (3)		[23] 7P (3)
	0,51	G	10.0.0.0.	0.8.0.0. (2)	0.1.5.0. (4)	0.0.1.4. (5)	0.0.0.4. 1P (5)		[24] 4P (6)
5. Solanidin-3-*β*-D-		KG	10.0.0.0.	0.0.10.0.	0.0.0.10.	0.0.0.0. 5P		[10] 5L_4	

oidglykoside bzw. Aglykone aus *Solanum*- oder *Lycopersicon*-Arten

Alkaloidglykoside bzw. Aglykone aus *Solanum-* oder *Lycopersicon*-Arten

6/55									
7. Solanidin-3-L-rhamnopyranosid-citrat (partialsynth.) 6/55		KG	10.0.0.0.	0.0.10.0.	0.0.0.10.	0.0.0.0. 5P		[10] 5L$_4$	
	0,26	G	10.0.0.0.	0.0.10.0.	0.0.0.10.				
	0,76	G	9.1.0.0.	0.0.10.0.	0.0.0.10.				
8. Solanidin-3-β-D-xylopyranosid-citrat (partialsynth.) 6/55		KG	10.0.0.0.	0.0.10.0.	0.0.0.10.	0.0.0.0. 5P		[10] 5L$_4$	
	0,23	G	10.0.0.0.	0.0.9.0. (1)	0.0.0.9. (1)				
	0,73	G	10.0.0.0.	0.0.9.0. (1)	0.0.0.4. (1)	0.0.0.0. 4P (1)		[9] 5L$_4$	
9. Solanidin-3-β-lactosid-(partialsynth.) 6/55		KG	10.0.0.0	0.0.10.0.	0.0.0.10.	0.0.0.0. 5P		[10] 5L$_4$	
	0,22	G	10.0.0.0.	0.1.8.0. (1)	0.0.0.9. (1)	0.0.0.0. 4P (2)		[10] 4L$_4$	
10. Dihydro-α-solanin-hydrochlorid (partialsynth.) 7/53		KG	10.0.0.0.	0.0.8.0. (2)	0.0.0.6. (4)	0.0.0.3. 2P (5)			[16] 5P (5)
	0,83	G	10.0.0.0.	0.4.5.0. (1)	0.1.2.5. (2)	0.0.0.4. (6)			[18] 3P (7)
11. Solacaulin-hydrochlorid (aus *S. acaule*) 6—8/55		KG	5.5.0.0.	0.0.10.0.	0.0.0.0. 5P			[9] 5L$_4$	
	0,20	G	4.6.0.0.	0.0.10.0.	0.0.0.5.	0.0.0.0. 4P (1)		[8] 5L$_4$	
	0,25	G	8.2.0.0.	0.1.9.0.	0.0.1.2. (2)	0.0.0.1. 1P (3)	0.0.0.1. 1P (3)	[9] 5L$_4$	[34] 2P (3)
	0,74	G	10.0.0.0.	1.6.1.0. (2)	0.3.2.1. (4)	0.0.1.2. (7)	0.0.1.2. (7)		[31] 1P (9)
12. Alkaloidtetraosid (freie Base) (aus *S. demissum*) 9/55		KG	8.1.0.0. (1)	0.0.9.0. (1)	0.0.1.3. (1)	0.0.0.1. 1P (3)		[8] 5L$_4$	[17] 2P (3)
	0,36	G	8.2.0.0.	0.6.3.0. (1)	0.0.3.0. (7)	0.0.0.1. (9)			[18] 1P (9)
	0,88	G	10.0.0.0.	2.1.0.0. (7)	0.2.0.0. (8)	0.1.1.0. (8)	0.0.0.0. (10)		
13. Alkaloidtetraosid-citrat (aus *S. demissum*) 8/55		KG	5.5.0.0.	0.0.10.0.	0.0.0.5.			[8] 5L$_4$	[14] 5P
	0,57	G	10.0.0.0.	1.8.0.0. (1)	0.1.1.0. (8)	0.0.0.0. (10)			
	0,90	G	10.0.0.0.	0.5.0.0. (5)	0.0.0.0. (10)				
14. Demissidin-acetat (partialsynth. aus Solanidin) 9/53		KG	8.0.0.0. (2)	0.8.0.0. (2)	0.1.6.0. (3)	0.0.0.5. (5)	0.0.0.0. 5P (5)		
	0,43	G	10.0.0.0.	4.4.0.0. (2)	1.6.1.0. (2)	0.0.7.0. (3)	0.0.0.3. (7)		[31] (10)
	0,44	G	10.0.0.0.	0.7.0.0. (3)	0.5.2.0. (3)	0.0.4.2. (4)	0.0.0.2. (8)		[23] (10)
15. Demissidin-3-β-D-glucopyranosid-citrat (partialsynth.) 6/55		KG	10.0.0.0.	0.0.10.0.	0.0.0.1. 3P (1)			[9] 5L$_4$	
	0,71	G	9.1.0.0.	0.0.10.0.	0.0.0.5.	0.0.0.1. 4P	0.0.0.1. 4P	[9] 5L$_4$	[25] 4P (1)
16. Polyadenin-hydrochlorid (aus *S. polyadenium*) 6/55		KG	1.9.0.0.	0.1.8.0. (1)	0.0.1.3. (1)		0.0.0.0. 3P (2)	[8] 5L$_4$	
	0,19	G	0.10.0.0.	0.1.9.0.	0.0.2.3.			[8] 5L$_4$	[14] 3P (2)
	0,40	G	0.10.0.0.	0.1.9.0.	0.1.0.4.	0.0.0.1. 2P (2)		[8] 5L$_4$	[19] 2P (3)
	0,52	G	10.0.0.0.	1.9.0.0.	0.0.8.0. (2)		0.0.0.8. (2)		[46] 6P (4)
17. Tomatin-citrat (aus *S. polyadenium*) 3—4/57		KG	7.3.0.0.	0.1.9.0.	0.0.0.4. (2)			[7] 2L$_3$ 2L$_4$	[13] 4P (2)
	0,24	G	9.1.0.0.	0.4.4.0. (2)	0.0.5.0 (5)	0.0.0.5. (5)	0.0.0.0. 5P (5)		[17] 1P (9)
	0,35	G	9.1.0.0.	0.3.0.0. (7)	0.0.1.0. (9)	0.0.0.1. (9)			[7] (10)
	0,46	G	10.0.0.0.	0.1.0.0. (9)					

Stoffgruppe	Substanz			Stand der Larvenentwicklung an dem betreffenden Tage nach Versuchsbeginn					Bemerkungen	
	Name (Vorkommen)	Konzentration im Test		2.	6.	10.	15.	20.	am Tage [n] wurden an Larven entfernt	am Tage [n] waren vorhanden
	Normalentwicklung (t-Kammer, ca. 24°)	Kontrollen		8.2.0.0.	0.4.6.0.	0.0.2.7. (1)	0.0.0.1. 7P (2)	0.0.0.1. 7P (2)		
	Normalentwicklung (Zimmertemperatur)			8.2.0.0.	0.5.4.0. (1)	0.0.4.4. (2)	0.0.0.1. 7P (2)	0.0.0.1. 7P (2)		
	Hungertiere			10.0.0.0.	0.0.0.0. (10)					[5] 3L1 (7)
caloidglykoside bzw. Aglykone aus *Solanum*- oder *Lycopersicon*-Arten	18. Solasoninhydrochlorid		KG	4.6.0.0.	0.1.9.0.	0.0.0.5.			[9] 5L4	[14] 4P (1)
	(aus *S. sodomeum*)	0,19	G	8.2.0.0.	0.1.9.0.	0.0.0.4. (1)			[8] 5L4	[14] 3P (2)
	8/55	0,79	G	2.8.0.0.	0.0.10.0.	0.0.0.5.	0.0.0.0. 5P		[9] 5L4	
		1,26	G	7.3.0.0.	0.3.6.0. (1)	0.0.3.6. (1)	0.0.0.3. 1P (1)	0.0.0.0. 4P (1)	[11] 5L4	
	19. Solamarginhydrochlorid		KG	9.1.0.0.	0.1.9.0.	0.0.1.4.	0.0.0.1. 4P		[8] 5L4	[16] 5P
	(aus *S. sodomeum*)	0,18	G	9.0.0.0. (1)	0.4.5.0. (1)	0.0.1.2. (2)	0.0.0.3. (2)		[8] 5L4	[18] 3P (2)
	7/57	0,28	G	10.0.0.0.	0.0.10.0.	0.0.0.5.	0.0.0.0. 4P (1)		[8] 5L4	
	20. Solasodin (freie Base)		KE	10.0.0.0.	0.1.7.2.	0.0.3.2.	0.0.0.3. 2P		[8] 5L4	[24] 5P
	(aus *S. aviculare*)	0,12	E	10.0.0.0.	0.2.8.0.	0.0.0.5.	0.0.0.0. 5P		[7] 5L3	
	4—6/57	0,30	E	0.10.0.0.	0.5.4.0. (1)	0.0.6.2. (2)	0.0.1.1. 2P (4)	0.0.0.0. 3P (5)	[10] 2L4	
		0,62	E	9.0.0.0. (1)	4.5.0.0. (1)	0.7.2.0. (1)	0.1.7.1. (1)	0.0.0.5. 1P (4)		[31] 5P (5)
	21. Roh-Solanigrin-citrat		KG	6.4.0.0.	0.0.10.0.	0.0.0.4. (1)			[7] 5L3	[14] 3P (2)
	(Alkaloidglykosid-	0,28	G	8.2.0.0.	0.4.5.0. (1)	0.0.0.5. (3)	0.0.0.0. 4P (4)		[9] 2L4	
	Gemisch aus *S. nigrum*)	0,52	G	8.2.0.0.	0.4.2.0. (4)	0.0.2.2. (6)	0.0.0.2. 2P (6)			[18] 3P (7)
	3/57									
	22. α-, β-, γ -Soladulcin-		KG	4.6.0.0.	0.1.8.0. (1)	0.0.0.5. (2)	0.0.0.2. 3P (2)		[7] 3L3	[18] 5P (2)
	citrat	0,023	G	9.1.0.0.	0.3.7.0.	0.0.2.4. (1)	0.0.2.0. 4P (1)		[7] 3L3	[16] 4P (3)
	(Alkaloidglykosid-	0,11	G	3.7.0.0.	0.2.4.0. (4)	0.0.1.4. (5)	0.0.0.2. 3P (5)	0.0.0.0. 5P (5)		
	Gemisch aus *S. dulcamara*)	0,24	G	2.8.0.0.	0.0.2.0. (8)	0.0.0.2. (8)	0.0.0.1. 1P (8)	0.0.0.1. 1P (8)		[40] 1P (9)
	3/57	0,36	G	2.8.0.0.	0.3.0.0. (7)	0.0.1.0. (9)	0.0.0.1. (9)	0.0.0.0. 1P (9)		
		0,46	G	9.1.0.0.	0.2.3.0. (5)	0.0.3.0. (7)	0.0.0.1. (9)			[18] 1P (9)
	23. Tomatin-citrat (aus		KG	10.0.0.0.	0.1.8.0. (1)	0.0.2.3. (1)	0.0.0.3. 2P (1)		[9] 4L4	[18] 5P (1)
	Lycopersicon pimpinelli-	0,09	G	10.0.0.0.	0.0.9.1.	0.0.3.2. (1)	0.0.0.1. 4P (1)		[9] 4L4	[16] 4P (2)

Alkaloide aus weiteren Solanaceen

4—6/57	0,28	E	6.4.0.0.	0.10.0.0.	0.1.5.2. (2)	0.0.0.2. 2P (4)		[12] 2L$_4$	[18] 4P (4)
	0,66	E	10.0.0.0.	2.7.1.0.	0.3.7.0.	0.0.3.5.	0.0.2.1. 2P (3)	[12] 2L$_4$	[45] 2P (6)
25. Atropinsulfat (aus		KG	10.0.0.0.	0.0.9.1.	0.0.0.5.			[8] 5L$_4$	[13] 5P
Atropa)	0,19	G	2.8.0.0.	0.1.8.1.	0.1.0.4.			[8] 5L$_4$	[13] 3P (2)
7/55 u. 6/56	0,53	G	1.9.0.0.	0.8.2.0.	0.0.6.3. (1)	0.0.0.5. (5)	0.0.0.1. 1P (8)		[22] 2P (8)
	0,94	G	4.6.0.0.	1.7.0.0. (2)	0.1.5.1. (3)	0.0.0.4. (6)	0.0.0.1. 1P (8)		[22] 2P (8)
26. Atropinmethylbromid		KG	3.7.0.0.	0.0.10.0.	0.0.0.5.	0.0.0.0. 5P		[8] 5L$_4$	
(partialsynth.)	0,23	G	0.10.0.0.	0.0.9.0. (1)	0.0.0.5. (1)	0.0.0.1. 4P		[8] 4L$_4$	[16] 5P (1)
7/56	1,07	G	7.3.0.0.	0.6.4.0.	0.0.9.0. (1)	0.0.8.0. (2)	0.0.0.5. (5)		[40] 1P (9)
27. Atropinmethylnitrat		KG	3.7.0.0.	0.0.10.0.	0.0.0.5.	0.0.0.0. 5P		[8] 5L$_4$	
(partialsynth.)	0,22	G	9.1.0.0.	0.2.8.0.	0.0.0.5.	0.0.0.0. 4P (1)		[9] 5L$_4$	
7/56	1,09	G	10.0.0.0.	0.8.0.0. (2)	0.3.4.0. (3)	0.0.4.1. (5)	0.0.0.5. (5)		
	2,41	G	8.0.0.0. (2)	4.1.0.0. (5)					[34] 3P (7)
28. Tropin (freie Base)		KG	9.1.0.0.	0.0.10.0.	0.0.0.5.			[7] 5L$_3$	[13] 5P
(aus Atropin)	0,26	G	2.8.0.0.	0.0.10.0.	0.0.0.4. (1)			[7] 5L$_4$	[12] 4P (1)
7 u. 8/56	0,65	G	9.1.0.0.	0.3.6.0. (1)	0.0.3.6. (1)	0.0.0.3. 1P (4)		[11] 2L$_4$	[17] 4P (4)
29. Homatropinhydrobromid		KG	10.0.0.0.	0.0.10.0.	0.0.0.5.			[8] 5L$_4$	[13] 4P (1)
(partialsynth.)	0,20	G	8.1.0.0. (1)	0.7.1.0. (2)	0.4.3.0. (3)	0.0.0.2. (8)			[18] 2P (8)
6/56	1,11	G	9.0.0.0. (1)	0.8.0.0. (2)	0.4.0.0. (6)	0.1.0.0. (9)			[18] (10)
30. Scopolaminhydrochlorid		KG	10.0.0.0.	0.0.10.0.	0.0.0.5.			[8] 5L$_4$	[13] 4P (1)
(aus *Datura*)	0,18	G	10.0.0.0.	0.8.0.0. (2)	0.1.6.1. (2)	0.0.0.5. 1P (4)			[17] 5P (5)
6/56	1,00	G	9.0.0.0. (1)	1.3.1.0. (5)	0.1.3.0. (6)	0.0.0.3. (7)	0.0.0.2. 1P (7)		[22] 1P (9)
	1,61	G	7.0.0.0. (3)	1.1.0.0. (8)					[8] (10)
31. Nicotin (freie Base)		KG	10.0.0.0.	0.0.10.0.	0.0.0.9. (1)				[14] 9P (1)
(aus *Nicotiana*)	0,0015	G	10.0.0.0.	0.6.2.0. (2)	0.1.2.3. (4)	0.0.0.2. 3P (5)			[17] 5P (5)
2—3/56	0,003	G	10.0.0.0.	0.9.1.0.	0.0.8.0. (2)	0.0.2.6. (2)	0.0.0.1. 4P (5)		[22] 5P (5)
	0,0075	G	2.0.0.0. (8)	0.0.0.0. (10)					
	0,03	G							[1] (10)
32. Nicotinhydrochlorid		KG	10.0.0.0.	0.0.10.0.	0.0.0.5.			[10] 5L$_4$	[13] 5P
(aus *Nicotiana*)	0,0023	G	10.0.0.0.	0.3.7.0.	0.0.1.9.	0.0.0.1. 4P		[10] 5L$_4$	[16] 5P
5/56	0,012	G	3.0.0.0. (7)						[4] (10)
	0,026	G	2.0.0.0. (8)						[4] (10)
33. Nicotincitrat		KG	10.0.0.0.	0.0.10.0.	0.0.0.10.			[10] 5L$_4$	[13] 5P
(aus *Nicotiana*)	0,0013	G	10.0.0.0.	0.1.9.0.	0.0.1.9.	0.0.0.0. 5P		[10] 5L$_4$	
5/56	0,003	G	10.0.0.0.	0.0.10.0.	0.0.0.9. (1)			[10] 4L$_4$	[13] 4P (2)
	0,029	G	2.0.0.0. (8)	0.0.0.0. (10)					

Stoffgruppe	Substanz		Stand der Larvenentwicklung an dem betreffenden Tage nach Versuchsbeginn					Bemerkungen	
	Name (Vorkommen)	Konzentration im Test	2.	6.	10.	15.	20.	am Tage [n] wurden an Larven entfernt	am Tage [n] waren vorhanden
	Normalentwicklung (t-Kammer, ca. 24°)	Kontrollen	8.2.0.0.	0.4.6.0.	0.0.2.7. (1)	0.0.0.1. 7P (2)	0.0.0.1. 7P (2)		
	Normalentwicklung (Zimmertemperatur)		8.2.0.0.	0.5.4.0. (1)	0.0.4.4. (2)	0.0.0.4. 4P (2)	0.0.0.1. 7P (2)		
	Hungertiere		10.0.0.0.	0.0.0.0. (10)					[5] $3L_1$ (7)
Alkaloide aus Pflanzen anderer Familien	34. Colchicin	KG	10.0.0.0.	0.0.9.0. (1)	0.0.0.8. (2)			[10] $3L_4$	[13] 5P (2)
	(freie Base)	0,0016 G	4.0.0.0. (6)	0.2.0.0. (8)	0.1.0.0. (9)				[11] (10)
	(aus *Colchicum*)	0,0025 G	6.0.0.0. (4)	0.0.0.0. (10)					
	5/56	0,0091 G	2.0.0.0. (8)						[4] (10)
		0,050 G	0.0.0.0. (10)						
	35. Colchicinhydrochlorid	KG	10.0.0.0.	0.0.9.0. (1)	0.0.0.8. (2)			[10] $3L_4$	[13] 5P (2)
	5/56	0,0017 G	10.0.0.0.	0.2.1.0. (7)	0.0.3.0. (7)				[13] (10)
		0,0034 G	6.0.0.0. (4)	0.0.0.0. (10)					
	36. Ephedrinhydrochlorid	KC	3.7.0.0.	0.0.8.0. (2)	0.0.0.5. (3)			[9] $2L_4$	[14] 5P (3)
	(aus *Ephedra*)	0,23 G	10.0.0.0.	0.1.9.0.	0.0.0.5. (1)	0.0.0.5. (1)		[9] $4L_4$	[18] 4P (2)
	7/56	0,44 G	10.0.0.0.	0.4.5.0. (1)	0.0.4.2. (4)	0.0.1.1. (8)	0.0.0.0. (10)		
		0,90 G	2.0.0.0. (8)	0.0.0.0. (10)					
	37. Pervitinhydrochlorid	KE	6.3.0.0. (1)	0.1.8.0. (1)	0.0.3.2. (1)	0.0.0.3. 1P (2)		[8] $4L_4$	[17] 4P (2)
	(synth.)	0,10 E	10.0.0.0.	0.2.8.0.	0.1.6.3.	0.0.0.2. 2P (6)	0.0.0.0. 3P (7)		
	5/57								
	38. Piperin (Säureamid)	KE	9 1.0.0.	0.3.4.0. (3)	0.0.2.5. (3)	0.0.0.0. 5P (3)		[11] $2L_4$	
	(aus *Piper*)	0,13 E	2.0.0.0. (8)						[5] (10)
	4/57	0,22 E	2.0.0.0. (8)						[4] (10)
	39. Physostigminsalicylat	KG	6.4.0.0.	0.1.9.0.					[8] $2L_3$ $8L_4$
	(aus *Physostigma*)	0,0011 G	5.0.0.0. (5)	0.1.0.0. (9)					[8] (10)
	7/56	0,0018 G	0.0.0.0. (10)						
		0,0163 G							[1] (10)
	40. Cocainhydrochlorid	KG	7.3.0.0.	0.0.10.0.	0.0.0.5. (2)			[8] $3L_4$	[13] 5P (2)
	(aus *Erythroxylon*)	0,02 G	10.0.0.0.	1.6.0.0. (3)	0.2.3.0. (5)	0.0.0.1. (9)	0.0.0.0. 1P (9)		

Alkaloide aus Pflanzen anderer Familien

43. Cinchonidinhydrogen-		KG	9.1.0.0.	0.0.10.0.	0.0.0.5.			[7] 5L_3	[13] 5P	
sulfat (aus *Cinchona*)	0,14	G	10.0.0.0.	0.8.0.0. (2)	0.0.6.0. (4)	0.0.0.2. (8)	0.0.0.1. (9)		[26] 1P	(9)
7/56	0,27	G	9.0.0.0. (1)	0.4.0.0. (6)	0.0.1.0. (9)	0.0.0.1. (9)			[19] 1P	(9)
	0,48	G	7.3.0.0.	0.2.0.0. (8)	0.1.0.0. (9)	0.0.0.0. (10)				
44. Chininhydrochlorid		KG	0.10.0.0.	0.1.9.0.	0.0.1.4.			[8] 2L_4	[14] 5P	
(aus *Cinchona*)	0,19	G	1.8.0.0. (1)	0.8.1.0. (1)	0.0.8.1. (1)	0.0.2.4. (1)	0.0.0.2. 1P (4)	[12] 3L_4	[27] 1P	(6)
7/55	0,40	G	1.7.0.0. (2)	0.7.0.0. (3)	0.2.4.0. (4)	0.1.3.2. (4)	0.0.0.2. (8)		[40]	(10)
	0,66	G	3.7.0.0.	0.3.0.0. (7)	0.3.0.0. (7)	0.0.0.0. (10)				
45. Papaverinhydrochlorid		KG	10.0.0.0.	0.0.10.0.	0.0.0.5.			[8] 5L_4	[13] 4P	(1)
(aus *Papaver*)	0,02	G	10.0.0.0.	0.0.9.0. (1)	0.0.0.5. (1)	0.0.0.1. 4P (1)		[8] 4L_4	[16] 5P	(1)
6/56	0,11	G	10.0.0.0.	0.5.2.0. (3)	0.0.5.1. (4)	0.0.0.5. (5)	0.0.0.3. 2P (5)		[39] 3P	(7)
	0,21	G	9.0.0.0. (1)	0.6.2.0. (2)	0.0.6.0. (4)	0.0.0.5. (5)	0.0.0.4. (6)		[39] 2P	(8)
	0,45	G	6.0.0.0. (4)	2.2.0.0. (6)	0.1.1.0. (8)	0.0.1.0. (9)			[17]	(10)
46. Narceinhydrochlorid		KG	9.1.0.0.	0.0.10.0.	0.0.0.4. 1P	0.0.0.1. 4P		[8] 5L_4	[17] 5P	
(aus *Papaver*)	0,25	G	6.4.0.0.	0.0.9.0. (1)	0.0.0.4. (2)	0.0.0.1. 2P (3)		[8] 4L_4	[18] 3P	(3)
8/56 u. 5/57		KE	10.0.0.0.	0.1.7.2.	0.0.3.2.	0.0.0.3. 2P		[8] 5L_4	[25] 5P	
	0,16	E	4.6.0.0.	0.1.9.0.	0.0.1.4.	0.0.0.0. 5P		[8] 2L_3 3L_4		
47. Narcotinhydrochlorid		KG	7.3.0.0.	0.0.10.0.	0.0.0.3. 2P			[7] 5L_4	[13] 4P	(1)
(aus *Papaver*)	0,02	G	3.7.0.0.	0.0.10.0.	0.0.0.8. (2)	0.0.0.0. 4P (4)		[11] 2L_4		
8/56	0,10	G	10.0.0.0.	0.4.5.0. (1)	0.0.6.1. (3)	0.0.0.1. (9)			[18]	(10)
	0,18	G	10.0.0.0.	0.6.3.0. (1)	0.0.1.0. (9)				[11]	(10)
48. Morphinhydrochlorid		KG	7.3.0.0.	0.0.10.0.	0.0.0.5. (2)			[8] 3L_4	[13] 5P	(2)
(aus *Papaver*)	0,11	G	10.0.0.0.	0.2.8.0.	0.0.3.7.	0.0.1.1. 5P		[10] 3L_4	[17] 6P	(1)
8/56	0,40	G	10.0.0.0.	0.2.8.0.	0.0.0.8. (2)			[10] 3L_4	[13] 5P	(2)
49. Codeinphosphat		KG	10.0.0.0.	0.2.6.0. (2)	0.0.1.4. (2)			[9] 3L_4	[14] 3L_4 2P	(2)
(partialsynth.)	0,10	G	4.0.0.0. (6)	0.0.4.0. (6)	0.0.0.4. (6)				[15] 4P	(6)
7/56	0,18	G	2.0.0.0. (8)	0.2.0.0. (8)	0.0.1.1. (8)				[12]	(10)
	0,39	G	0.0.0.0. (10)							
50. Thebainhydrochlorid		KG	10.0.0.0.	0.1.5.0. (4)	0.0.0.6. (4)	0.0.0.0. 6P (4)			[17] 1P	(9)
(aus *Papaver*)	0,03	G	10.0.0.0.	0.1.8.0. (1)	0.0.0.3. (7)	0.0.0.1. (9)			[8]	(10)
8—9/56	0,10	G	10.0.0.0.	0.5.0.0. (5)						
51. Oxyacanthinhydrochlorid		KG	9.1.0.0.	0.0.10.0.	0.0.0.4. 1P	0.0.0.1. 4P		[8] 5L_4	[17] 5P	
(aus *Berberis*) 8/56	0,21	G	8.2.0.0.	0.0.9.0. (1)	0.0.1.7. (2)	0.0.0.3. 1P (4)	0.0.0.2. 2P (4)	[11] 2L_4	[35] 4P	(4)
52. Curinhydrochlorid		KG	7.3.0.0.	0.0.10.0.	0.0.0.3. 2P			[7] 5L_4	[13] 4P	(1)
(aus *Chondodendron*)	0,18	G	8.2.0.0.	0.3.7.0.	0.0.1.7. (2)	0.0.0.4. 1P (3)	0.0.0.1. 3P (4)	[10] 2L_4	[24] 3P	(5)
8/56										

Stoffgruppe	Substanz			Stand der Larvenentwicklung an dem betreffenden Tage nach Versuchsbeginn					Bemerkungen	
	Name (Vorkommen)	Konzentration im Test		2.	6.	10.	15.	20.	am Tage [n] wurden an Larven entfernt	am Tage [n] waren vorhanden
	Normalentwicklung (t-Kammer, ca. 24°)	Kontrollen		8.2.0.0.	0.4.6.0.	0.0.2.7. (1)	0.0.0.1. 7P (2)	0.0.0.1. 7P (2)		
	Normalentwicklung (Zimmertemperatur)			8.2.0.0.	0.5.4.0. (1)	0.0.4.4. (2)	0.0.0.4. 4P (2)	0.0.0.1. 7P (2)		
	Hungertiere			10.0.0.0.	0.0.0.0. (10)					[5] 3L1 (7)
Alkaloide aus Pflanzen anderer Familien	53. Berberin (freie Base)		KG	9.1.0.0.	0.0.10.0.	0.0.0.4. 1P	0.0.0.1. 4P		[8] 5L4	[17] 5P
	(aus *Berberis*)	0,08	G	10.0.0.0.	0.4.0.0. (6)	0.1.3.0. (6)	0.1.3.0. (6)	0.0.4.0. (6)		[27] (10)
	8/56	0,18	G	6.0.0.0. (4)	1.3.0.0. (6)	0.3.0.0. (7)	0.2.0.0. (8)			[16] (10)
	54. Corydalinhydrochlorid		KG	1.9.0.0.	0.1.8.0. (1)	0.0.0.5. (2)			[9] 3L4	[13] 4P (3)
	(aus *Corydalis*)	0,01	G	5.5.0.0.	0.2.5.0. (3)	0.0.0.4. 1P (5)				[13] 4P (6)
	8/56	0,14	G	10.0.0.0.	0.2.0.0. (8)					[9] (10)
		0,21	G	10.0.0.0.	1.2.0.0. (7)					[8] (10)
	55. Emetinhydrochlorid		KG	7.3.0.0.	0.0.10.0	0.0.0.5. (2)			[8] 3L4	[13] 5P (2)
	(aus *Uragoga*)	0,02	G	9.1.0.0.	0.7.1.0. (2)	0.2.1.0. (7)	0.0.1.0. (9)	0.0.0.0. (10)		
	8/56	0,19	G	10.0.0.0.						[4] (10)
	56. Chelidoninsulfat		KG	9.1.0.0.	0.0.10.0.	0.0.0.4. 1P	0.0.0.1. 4P	0.0.0.1. 1P (8)	[8] 5L4	[17] 5P
	(aus *Chelidonium*)	0,21	G	10.0.0.0.	0.7.2.0. (1)	0.1.3.2. (4)	0.0.0.2. (8)			[29] 1P (9)
	8/56									
	57. Sanguinarinsulfat		KG	1.9.0.0.	0.1.8.0. (1)	0.0.0.5. (2)			[9] 3L4	[13] 4P (3)
	(aus *Chelidonium*)	0,01	G	7.3.0.0.	0.0.9.0. (1)	0.0.0.5. (1)			[8] 4L4	[13] 5P (1)
	8/56	0,02	G	9.1.0.0.	0.7.2.0. (1)	0.0.5.2. (3)	0.0.1.3. 1P (5)	0.0.0.3. 2P (5)		[23] 4P (6)
		0,12	G	10.0.0.0.	0.1.0.0. (9)					[7] (10)
	58. Yohimbinhydrochlorid		KG	9.1.0.0.	0.0.10.0.	0.0.0.4 1P	0.0.0.1. 4P		[8] 5L4	[17] 5P
	(aus *Corynanthe*)	0,20	G	10.0.0.0.	0.0.10.0.	0.0.2.6, (2)	0.0.0.4. (6)	0.0.0.1. 2P (7)		[23] 3P (7)
	8/56									
	59. Strychnincitrat		KG	10.0.0.0.	0.0.10.0.	0.0.0.5. (2)			[8] 5L4	[13] 4P (1)
	(aus *Strychnos*)	0,01	G	10.0.0.0.	0.0.6.0. (4)	0.0.0.5. (5)				[13] 4P (6)
	6/56	0,02	G	9.1.0.0.	0.4.6.0.	0.0.5.2. (3)	0.0.0.1. 1P (8)	0.0.0.0. 1P (9)		
		0,03	G	8.0.0.0. (2)	0.5.0.0. (5)	0.0.0.0. (10)				
		0,11	G	9.0.0.0. (1)	0.0.0.0. (10)					
	59. Brucinhydrochlorid		KG	9.1.0.0.	0.0.10.0.	0.0.0.5			[9] 5L3	[13] 5P

	6/56	0,10	G	10.0.0.0.	0.7.2.0. (1)	0.0.1.2. (7)	0.0.1.2. (7)	0.0.0.1. 2P (7)		[31] 5P	(7)
		0,23	G	10.0.0.0.	0.10.0.0.	0.1.2.0. (7)	0.0.0.1. (9)			[17]	(10)
Alkaloide aus Pflanzen anderer Familien	62. Theobromin (freie		KE	9.1.0.0.	0.3.4.0. (3)	0.0.2.5. (3)	0.0.0.0. 5P (3)		[11] $2L_4$		
	Base) (aus *Theobroma*)	0,12	E	10.0.0.0.	0.8.2.0.	0.1.8.1.	0.0.0.6. (1)	0.0.0.2. 2P (3)	[13] $3L_4$	[21] 4P	(3)
	4—5/57	0,30	E	5.5.0.0.	0.10.0.0.	0.2.5.0. (3)	0.0.2.3. (5)	0.0.2.2. (6)			
	63. Veratrinhydrochlorid		KG	9.1.0.0.	0.0.10.0.	0.0.0.4. 1P	0.0.0.1. 4P		[8] $5L_4$	[17] 5P	
	(aus *Schoenocaulon*)	0,0018	G	6.0.0.0. (4)	2.1.0.0. (7)	0.0.0.0. (10)					
	8/56	0,0093	G	2.0.0.0. (8)	2.0.0.0. (8)					[8]	(10)
		0,0131	G	1.0.0.0. (9)						[3]	(10)
		0,0930	G	0.0.0.0. (10)							
	64. Sabadillin (freie Base)		KE	6.3.0.0. (1)	0.1.8.0. (1)	0.0.3.2. (1)	0.0.0.3. 1P (2)		[8] $4L_4$	[17] 4P	(2)
	(aus *Schoenocaulon*)	0,18	E	9.1.0.0.	0.4.1.0. (5)	0.1.2.0. (7)	0.0.1.1. (8)	0.0.0.2. (8)		[24] 1P	(9)
	5/57										
	65. Aconitinsulfat		KG	7.3.0.0.	0.0.10.0.	0.0.0.5. (2)			[8] $3L_4$	[13] 5P	(2)
	(aus *Aconitum*)	0,003	G	10.0.0.0.	0.6.4.0.	0.1.3.5. (1)	0.0.0.0. 4P (4)		[11] $2L_4$		
	8/56	0,014	G	9.0.0.0. (1)	0.3.0.0. (7)					[8]	(10)
		0,023	G	6.0.0.0. (4)	0.0.0.0. (10)						
	66. Delphininhydrochlorid		KG	10.0.0.0.	0.1.5.0. (4)	0.0.0.6. (4)	0.0.0.0. 6P (4)				
	(aus *Delphinium*)	0,0018	G	7.0.0.0. (3)	0.0.0.0. (10)						
	9/56	0,0168	G	7.0.0.0. (3)	0.0.0.0. (10)						
Saponine u. chem. verwandte Verbindungen	67. Digitonin		KG	10.0.0.0.	0.0.10.0.	0.0.0.0. 5P			[8] $5L_4$		
	(aus *Digitalis*)	0,22	G	9.1.0.0.	0.5.1.0. (4)	0.0.3.0. (7)	0.0.0.2. (8)	0.0.0.0. 2P (8)		[24] 2P	(8)
	(Saponin)	0,37	G	8.0.0.0. (2)	0.1.0.0. (9)	0.0.1.0. (9)	0.0.0.1. (9)	0.0.0.0. 1P (9)			
	6/56	0,77	G	9.0.0.0. (1)	0.1.0.0. (9)	0.0.0.0. (10)					
	68. Saponinum album		KG	0.10.0.0.	0.0.8.2.	0.0.0.5.			[9] $5L_4$	[14] $1L_4$ 4P	
	„Merck"	0,25	G	8.0.0.0. (2)	1.0.0.0. (9)					[8]	(10)
	8/55	0,48	G	8.1.0.0. (1)						[4]	(10)
	69. Saponinum album		KG	5.5.0.0.	0.2.8.0.	0.0.1.4.			[9] $5L_4$	[12] $5L_4$	
	„Riedel"	0,25	G	7.0.0.0. (3)	0.0.0.0. (10)						
	8/55	0,43	G	8.0.0.0. (2)	0.0.0.0. (10)						
	70. Sapotoxin		KG	3.7.0.0.	0.0.10.0.	0.0.0.3. 2P			[7] $3L_3$ $2L_4$	[12] 5P	
	(Saponin)	0,02	G	1.9.0.0.	0.3.0.0. (7)	0.0.1.0. (9)	0.0.0.1. (9)	0.0.0.0. (10)			
	7—8/56	0,22	G	9.0.0.0. (1)	0.0.1.0. (9)	0.0.0.0. (10)					
		0,40	G	6.0.0.0. (4)						[4]	(10)
	71. Convallamarin		KG	3.7.0.0.	0.0.10.0.	0.0.0.3. 2P			[7] $3L_3$ $2L_4$	[12] 5P	
	(aus *Convallaria*)	0,102	G	6.4.0.0.	0.0.7.0. (3)	0.0.0.5. (3)			[9] $2L_4$	[14] 5P	(3)
	(Glykosid)	0,200	G	8.0.0.0. (2)	0.0.7.0. (3)	0.0.0.4. (6)				[14] 4P	(6)
	7—8/56	0,504	G	8.0.0.0. (2)	0.5.1.0. (4)	0.0.1.0. (9)	0.0.0.1. (9)			[18] 1P	(9)

Stoffgruppe	Substanz			Stand der Larvenentwicklung an dem betreffenden Tage nach Versuchsbeginn					Bemerkungen	
	Name (Vorkommen)	Konzentration im Test		2.	6.	10.	15.	20.	am Tage [n] wurden an Larven entfernt	am Tage [n] waren vorhanden
	Normalentwicklung (t-Kammer, ca. 24°)	Kontrollen		8.2.0.0.	0.4.6.0.	0.0.2.7. (1)	0.0.0.1. 7P (2)	0.0.0.1. 7P (2)		
	Normalentwicklung (Zimmertemperatur)			8.2.0.0.	0.5.4.0. (1)	0.0.4.4. (2)	0.0.0.4. 4P (2)	0.0.0.1. 7P (2)		
	Hungertiere			10.0.0.0.	0.0.0.0. (10)					[5] $3L_1$ (7)
Saponine u.a.	72. Cholesterin		KG	1.9.0.0.	0.0.9.0. (1)	0.0.0.7. (3)	0.0.0.0. 7P (3)			
	(Zoosterin)	0,29	G	1.9.0.0.	0.1.9.0.	0.1.0.5.			[9] $4L_4$	[14] 4P (2)
	8/55	0,48	G	0.10.0.0.	0.0.10.0.	0.0.0.5.			[9] $5L_4$	[13] 5P
	73. Desoxycholsäure		KG	8.2.0.0.	0.0.10.0.	0.0.1.4. (1)	0.0.0.1. 4P (1)		[9] $4L_4$	[16] 4P (2)
	(Na-Salz) (Gallensäure)	0,211	G	8.2.0.0.	0.2.8.0.	0.0.1.5. (2)	0.0.0.2. 2P (4)		[9] $2L_4$	[17] 4P (4)
	3/57	0,444	G	10.0.0.0.	0.1.5.0. (4)	0.0.1.3. (6)	0.0.0.3. (7)	0.0.0.0. 2P (8)		
Weitere Verbindungen	74. Aesculin (aus *Aesculus*)		KE	10.0.0.0.	0.1.7.2.	0.0.3.2. (1)	0.0.0.3. 2P	0.0.0.1. 4P	[8] $5L_4$	[25] 5P
	(Glykosid) 5/57	0,16	E	8.2.0.0.	0.1.9.0.	0.0.1.4. (1)			[8] $2L_3$ $2L_4$	[14] 5P (1)
	75. Ononin (aus *Ononis*)		KE	6.3.0.0. (1)	0.1.8.0. (1)	0.0.3.2. (1)	0.0.0.3. 1P (2)		[8] $4L_4$	[17] 4P (2)
	(Glykosid) 5/57	0,16	E	10.0.0.0.	1.1.8.0.	0.0.4.5. (1)	0.0.1.2. 2P (2)	0.0.0.1. 4P (2)	[10] $3L_4$	[24] 5P (2)
	76. Phloridzin (aus Rosaceen)		KE	6.3.0.0. (1)	0.1.8.0. (1)	0.0.3.2. (1)	0.0.0.3. 1P (2)		[8] $4L_4$	[17] 4P (2)
	(Glykosid) 5/57	0,12	E	5.5.0.0.	0.1.8.0. (1)	0.0.0.6. (1)			[8] $3L_4$	[14] 6P (1)
	77. Elaterin (aus *Ecballium*)		KE	10.0.0.0.	0.1.7.2.	0.0.3.2.	0.0.0.3. 2P	0.0.0.1. 4P	[8] $5L_4$	[25] 5P
	(Bitterstoff) 5/57	0,17	E	7.0.0.0. (3)	0.3.3.0. (4)	0.2.2.2. (4)	0.0.0.0. 4P (6)			
	78. Peucedanin		KE	9.0.0.0. (1)	1.6.2.0. (1)	0.2.5.1. (2)	0.0.1.3. (4)	0.0.0.1. 1P (6)	[13] $2L_4$	[24] 2P (8)
	(aus *Peucedanum*)	0,15	E	9.0.0.0. (1)	0.5.2.0. (3)	0.2.5.0. (3)	0.0.0.4. (6)	0.0.0.3. 1P (6)		[23] 2P (8)
	(Bitterstoff) 6/57									
	79. Picrotoxin (aus *Anamirta*)		KE	9.1.0.0.	0.3.4.0. (3)	0.0.2.5. (3)	0.0.0.0. 5P (3)		[11] $2L_4$	
	(Bitterstoff) 4/57	0,09	E	10.0.0.0.	3.1.0.0. (6)	1.0.1.0. (8)				[13] (10)
	80. Quassiin (aus *Quassia*)		KE	6.3.0.0. (1)	0.1.8.0. (1)	0.0.3.2. (1)	0.0.0.3. 1P (2)		[8] $4L_4$	[17] 4P (2)
	(Bitterstoff) 5/57	0,13	E	6.0.0.0. (4)	6.0.0.0. (4)	1.0.0.0. (9)				[11] (10)
	81. Santonin		KE	9.1.0.0.	0.3.4.0. (3)	0.0.2.5. (3)	0.0.0.0. 5P (2)		[11] $2L_4$	
	(aus *Artemisia*)	0,11	E	10.0.0.0.	0.5.4.0. (1)	0.0.3.2. (1)	0.0.0.5 (1)	0.0.0.1. 4P (1)	[9] $2L_3$ $2L_4$	[21] 5P (1)
	4—5/57	0,37	E	9.1.0.0.	0.10.0.0.	0.0.9.0. (1)	0.0.4.3. (2)	0.0.1.1. (8)		[34] (10)
	82. Cubebin (aus *Piper*)		KE	10.0.0.0.	0.1.7.2.	0.0.3.2.	0.0.0.3. 2P	0.0.0.1. 4P	[8] $5L_4$	[25] 5P

gleichsinnig, nur mit betonterer Wirkung, liegen die Ergebnisse für Scopolamin (30), wogegen das Tropin (28) Störungen ähnlicher Art nicht erkennen liess.

Zur Veranschaulichung der Ergebnisse wurde der Einfluss, den besonders interessierende Solanaceen-Alkaloide auf den Entwicklungsgang der Larven ausüben, mit den Kurven der Abb. 1 dargestellt.

Die Wirkungsweise der Alkaloide aus Nicht-Solanaceen (34—66) und der geprüften weiteren Verbindungen (67—83) sei im Hinblick auf die hier in nur sehr gedrängter Form mögliche Darstellung der mannigfaltigen Befunde ebenfalls an Hand der Abb. 1 geschildert, wobei der Eingruppierung Konzentrationen zugrunde gelegt wurden, wie sie in der Abbildung für die Solanaceen-Alkaloide, auf welche Bezug genommen wird, angegeben sind.

In eine erste Gruppe von Stoffen, die wie etwa das Nicotin (Abb. 1 — □ —), schon in geringen Konzentrationen unmittelbar zu einem plötzlichen Sterben der Tiere führen, sind nach den Testungen folgende Alkaloide zu rechnen, deren Wirksamkeit in der angeführten Reihe abnimmt: Physostigmin (39), Colchicin (34, 35), Nicotin (31—33), Veratrin (63, vgl. 64), Aconitin (65) und Delphinin (66).

In ihrer Wirkungsstärke etwa dem Tomatin (Abb. 1 — • —) gleichzusetzen, ist die Mehrzahl der getesteten Stoffe, wobei dahingestellt sei, ob ihre Wirkungsweise vorwiegend eine vergällende oder eine toxische ist. Nach ihrer Wirksamkeit geordnet, gehören folgende Substanzen hierher: Piperin (38), Cocain (40), Emetin (55), Codein (49), Strychnin (59), Thebain (50), Sanguinarin (57), Corydalin (54), Berberin (53), Narcotin (47), Brucin (60), Coffein (61), Cinchonin (41, 42), Cinchonidin (43), Tomatin (17, 23), Papaverin (45) und Chelidonin (56).

Zu einer dritten Gruppe mit Stoffen intermediärer Wirkung (vgl. Abb. 1, Solacaulin — △ — und Atropin — ▼ —) wären folgende Alkaloide zu rechnen: Solacaulin (11), Scopolamin (30), Atropin und Derivate (25—27, 29), Ephedrin (36, vgl. 37), Chinin (44), Narcein (46), Curin (52), Yohimbin (58), Theobromin (62), Morphin (48) und Oxyacanthin (51), wobei die beiden zuletzt genannten Alkaloide kaum mehr wirksam waren.

Die Saponine (67—70) sind biogenetisch und chemisch mit den *Solanum*-Alkaloidglykosiden verwandt; auch weist ihre Wirkungsweise und -stärke auf die Larven des Kartoffelkäfers gewisse Parallelen zu der Wirkung des Tomatins (Abb. 1 — • —) auf (vgl. Schreiber, 1957, 1958). Ähnlich wirksam sind Convallamarin (71) und Desoxycholsäure (73).

Von den weiterhin getesteten Verbindungen erwiesen sich Cantharidin (83), Quassiin (80) und Pikrotoxin (79) als stark wirksam; intermediär verhielten sich Elaterin (77), Peucedanin (78) und Santonin (81), während Aesculin (74), Ononin (75) und Phloridzin (76) einen Einfluss auf die Larvenentwicklung nicht erkennen liessen.

SUMMARY

Eighty three alkaloids, saponins and some other naturally occurring substances and their derivatives were tested for their effect on the larvae of the Colorado potato beetle (*L. decemlineata* Say). Special attention was paid to the alkaloid-glycosides of some *Solanum* and *Lycopersicon* species, to further *Solanaceae*-alkaloids, and to some sapogenins, related to the *Solanum*-alkaloid glycosides in their biogenesis, chemistry and larval activity.

Whereas tomatin (for example) affected the larvae mainly by repellent action of the food, nicotin even in quite small concentrations had a pronounced toxic effect. It should be noted that physostigmin, colchicin,veratrin, aconitin, delphinin as well as cantharidin, quassiin and picrotoxin, like nicotin, caused sudden death of the larvae soon after their ingestion. A comparable effect to tomatin was also observed among the investigated *Solanum* alkaloid-glucosides with the tetraosides isolated from *Sol. demissum* and *Sol. polyadenium,* with the soladulcin mixture from *Sol. dulcamara,* similarly with a number of other alkaloids of very different origin and notably with the tested saponins and related substances. Further substances investigated, especially a number of *Solanum* glyco-alkaloids (e.g., solacaulin) and other Solanaceae alkaloids (e.g., atropin), showed an intermediate effect on the larvae; others again (e.g., alpha-solanin) had only a small or no influence on larval development.

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RELATION BETWEEN GROWTH PATTERN AND RESISTANCE TO *APHIS FABAE* SCOPOLI IN THREE VARIETIES OF FIELD BEAN (*VICIA FABA* L.).

BY

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Three varieties of Field (Spring) Bean were compared in a small plot trial: Rastatt (known to be relatively resistant to *Aphis fabae*), Schlanstedt (very susceptible) and an English Tick Bean. Records were kept of the growth of the plants and of the day-to-day incidence of winged *Aphis fabae* and *Acyrthosiphon pisum*, with periodic aphicidal spraying to minimize damage to the plants.

Schlanstedt was the first to flower and fruit, Rastatt intermediate and Tick last with the most shoots, leaves and flowers. Yields were about the same from Schlanstedt and Rastatt and somewhat lower from Tick. The relative incidence of *A. fabae* both among the organs of one variety and between varieties was correlated with growth pattern. The smaller number of aphids on Rastatt compared with both Schlanstedt and Tick was associated with the "compromise" growth pattern of Rastatt, wherein growth was more equally divided between shoots and fruits, and apparently less vigorous in the individual organs, during the critical weeks.

These observations, together with the contrasted behaviour of *A. pisum* and previous work on phloem sap composition in relation to plant growth and aphid nutrition, suggest that the sap may have been least nutritious for aphids in Rastatt, and that the resistance of Rastatt to *A. fabae* may have been due to the winged migrants responding to some superficial plant features associated with growth pattern and sap composition. Possible practical implications are briefly mentioned.

PAINTER (1958) states that resistance has been developed in crop plants far more often against aphids than against insects of any other group. Because resistance is no more frequent with aphid species restricted to a few kinds of plant than with species having a wide host range, he goes on to suggest that aphids are not after all so closely dependent on their host plants as their parasitic reputation would imply. This suggestion takes into account only the taxonomic status of the host. There is considerable evidence that aphid infestation depends also on the plant's current physiological status (DAVIDSON 1925; KENNEDY 1958). Thus a more plausible alternative explanation of the facts to which PAINTER refers would be that resistance commonly rests on physiological characters rather than on the taxonomic characters which may largely determine host range. A preliminary examination of this possibility in one well-established case of varietal resistance to *Aphis fabae* is described here.

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Since *A. fabae* feeds and larviposits more readily on growing and senescing plant organs than on mature ones, it follows that a difference between two plant species in the timing and pattern of growth, flowering and senescence can make one habitually more resistant than the other in the same environment. This is seen in extreme form in the host alternation of *A. fabae*. The woody winter host, *Euonymus europaeus* L., is resistant to the spring and summer migrants of *A. fabae* because most of its leaves are mature at that time and not because it is taxonomically unacceptable to them (KENNEDY & BOOTH, 1951, 1954). LINDEMANN (1948) and MITTLER (1953, 1958) have shown with other species of aphids and plants that the phloem sap is richer in organic nitrogenous compounds during growth and senescence than at maturity and recorded corresponding variations in the growth rate and behaviour of the aphids. Thus a difference of resistance even between varieties within one plant species could also be connected in some cases with a small difference of growth pattern affecting among other things the nutritiousness of the sap for the aphids. The work of TAYLOR (1955) and others on growth pattern and aphid resistance in potato varieties lends colour to this hypothesis; indeed DAVIDSON's (1922) original hypothesis to explain the results of his pioneer experiments on susceptibility to *A. fabae* among varieties of *Vicia faba* was similar, invoking physiological differences affecting sap nutritiousness. He recorded simply the multiplication rates of *A. fabae* when placed on potted plants. He did not study physiological differences between his bean varieties but based his hypothesis on the abundant field and laboratory evidence (including his own: DAVIDSON, 1925) of environmental effects on plant susceptibility to aphids. AUCLAIR and MALTAIS (see AUCLAIR, 1958) have now shown that varieties of peas susceptible to the pea aphid *Acyrthosiphon pisum* (Harris) usually contain more amino-nitrogen in the young growing shoots (phloem sap was not analysed) than resistant varieties, but this has not yet been related to growth patterns.

MÜLLER (1951, 1953, 1958) has rejected DAVIDSON's hypothesis for *A. fabae* without examining growth patterns or sap composition. Using mainly two varieties of Spring Field Bean, Schlanstedt and Rastatt (the most susceptible and most resistant available), he found a significantly greater fecundity in *A. fabae* on Schlanstedt but a much more pronounced active preference for Schlanstedt by winged migrants alighting in the field. Some of the aphids showed this preference after probing the leaf surface with their mouth-parts very superficially and inter-cellularly without penetrating the vacuole of any cell. It would seem that sap quality is not in any immediate sense the main cause of the resistance of Rastatt, and one implication in DAVIDSON's hypothesis has been disproved: that aphids are affected by plant-physiological variations exclusively through the sap quality governing their nutrition. But his general hypothesis stands, for variations as of growth pattern affect behaviour as well as nutrition. An association between superficial plant factors and sap quality has not been disproved, and active responses by the aphids to the former as "token" stimuli would make more nutritious varieties doubly susceptible. The next logical step was therefore to see if there was any difference of growth pattern between Rastatt and other varieties which might be related to their unequal susceptibility.

MATERIAL AND METHODS

Seed of Rastatt and Schlanstedt was obtained from the Institut für Pflanzenzüchtung, Quedlinburg, Germany, by courtesy of Dr. H. J. MÜLLER, and seed of an un-named commercial variety of English 'Tick' bean was kindly supplied by the University Farm, Cambridge. These were sown together with seed of *Vicia narbonensis* (N, below) and *Phaseolus vulgaris* (P) on April 4th, 1952, at the Entomological Field Station, Cambridge, in the layout shown below.

T	S	N	R	P	T	S	N	R	P
S	N	R	P	T	S	N	R	P	T
N	R	P	T	S	N	R	P	T	S
R	P	T	S	N	R	P	T	S	N
P	T	S	N	R	P	T	S	N	R

Two feet were left between each sowing position and the next to allow the necessary free access to the plants for recording purposes, and resulted in growth patterns rather different from those obtained in agricultural practice. The Tick Bean plants, in particular, tillered more than normally. Most of the *V. narbonensis* seed failed to germinate; records from this species and from *P. vulgaris* were discontinued to permit concentration of attention on the three varieties of *V. faba*, and will not be considered here. Five seeds of each variety were sown close together at each position to give more plant mass for aphids when the plants were small. On May 15th the plants were thinned to leave two vigorous specimens at each position. Growth recording and aphid counting were stated on April 29th.

The type of growth record made was determined largely by aphidological considerations. The height (length of longest shoot) of each plant was recorded as well as the number and combined length of shoots. The compact bunch of buds and unexpanded leaves at each growing shoot tip was counted as one unit, including developing inflorescences consisting of closely adpressed floret buds between which the alate aphids could not crawl. The nodes with expanded leaves were counted as individual units and classified according to the stage of the flowers and fruits, it any. Nodes with loosening floret buds between which alate aphids could crawl were distinguished from nodes at which the flowers had opened, and these in turn from nodes at which pods were visible. Nodes were counted as flower or pod nodes only so long as at least one flower or pod remained there, after which they were counted simply as expanded leaf nodes. The last growth records were made in July; on August 8th the plants were harvested, the total number of pods on each counted and the seeds then counted and weighed.

From May 15th to June 17th the winged *A. fabae* were counted on the plants almost daily, with a final count on June 25th. Only those alatae which were settled and apparently feeding were counted; many of these were in or between the furled leaves at the shoot tips or between the loosening floret buds, which were gently parted to find them. In an attempt to obtain some information on their reproduction as well as their incidence on the plants, the alatae were not removed at every count. But they and their progeny were all killed and washed off with HETP spray at intervals (Fig. 2) to prevent the production of second-generation progeny and minimize damage to the plants. On each of eleven days in June the alatae were counted separately on the various organs of the plants and removed at each count. Neither these removals nor the periodic sprayings made any obvious difference to the daily numbers of alatae found (Fig. 2). It appeared that most of those counted on one day had left again by the next (cp. Kennedy, 1950; Moericke, 1955; Müller, 1951, 1953) and most counted on each day were new arrivals. Apart from *A. fabae,* the only aphid species found on the plants as more than an occasional visitor, was *Acyrthosiphon pisum* (Harris). Alatae of *A. pisum* were counted on four days in June and their progeny on one (Fig. 2).

RESULTS

Plant Growth

Shoots. Schlanstedt (S) and Rastatt (R) emerged together and the Tick Beans (T) a few days later. The S and R plants grew much taller than the T plants (Table I). The S main shoots lengthened faster than R at first, being significantly longer on May 7 and 14 ($P < 0.01$); but in June R overtook S and by July R was significantly taller ($P < 0.001$). Tillering (Table I and Fig. 1) began on T in May, but not until June on S and R. R drew level with T in number of shoots in June but then T went on to produce many more side-shoots; S produced fewer than R. In combined length of shoots S was leading and T behind R through April and May, but R and T overtook S in June and T overtook R in July.

Table I

Shoot growth on the three varieties: Schlanstedt (S), Rastatt (R) and Tick (T). Means per plant.

	No. Shoots			Length of longest shoot, cm.			Combined length of shoots, cm.		
Date	S	R	T	S	R	T	S	R	T
29/4	1	1	1	5.9	5.6	3.6	5.9	5.6	3.6
7/5	1	1	1.1	12.0	10.2	6.5	12.0	10.2	6.3
14/5	1	1	1.3	19.3	16.3	10.5	19.3	16.5	10.5
23/5	1	1	2.0	31.8	30.2	19.3	31.8	30.9	28.9
4/6	2.5	2.4	3.3	52.7	53.6	33.9	68.8	72.8	75.9
13/6	3.4	4.0	4.0	70.1	76.7	48.4	133.6	148.1	139.6
10/7	4.1	5.5	9.0	103.4	125.0	88.9	247.1	435.5	521.0

Leaves (Fig. 1). From May 14 onwards, the T plants, with shorter inter-nodes and more shoots, produced expanded leaves faster than S and R, until by July T carried 50% more. R and S produced about the same number through April, May and June but then R went on to produce a significantly larger number of leaves ($P < 0.01$) in July.

Flowers and Fruits (Fig. 1.). The first flower buds developed at about the 8th node (above soil level) on the main shoots of S, about the 10th of R and about the 12th of T. Hence the "loose" flower buds became available earliest on S and latest on T. Nodes with such buds were significantly more numerous on S and R in the last week of May ($P < 0.01$) and first week of June ($P < 0.001$).

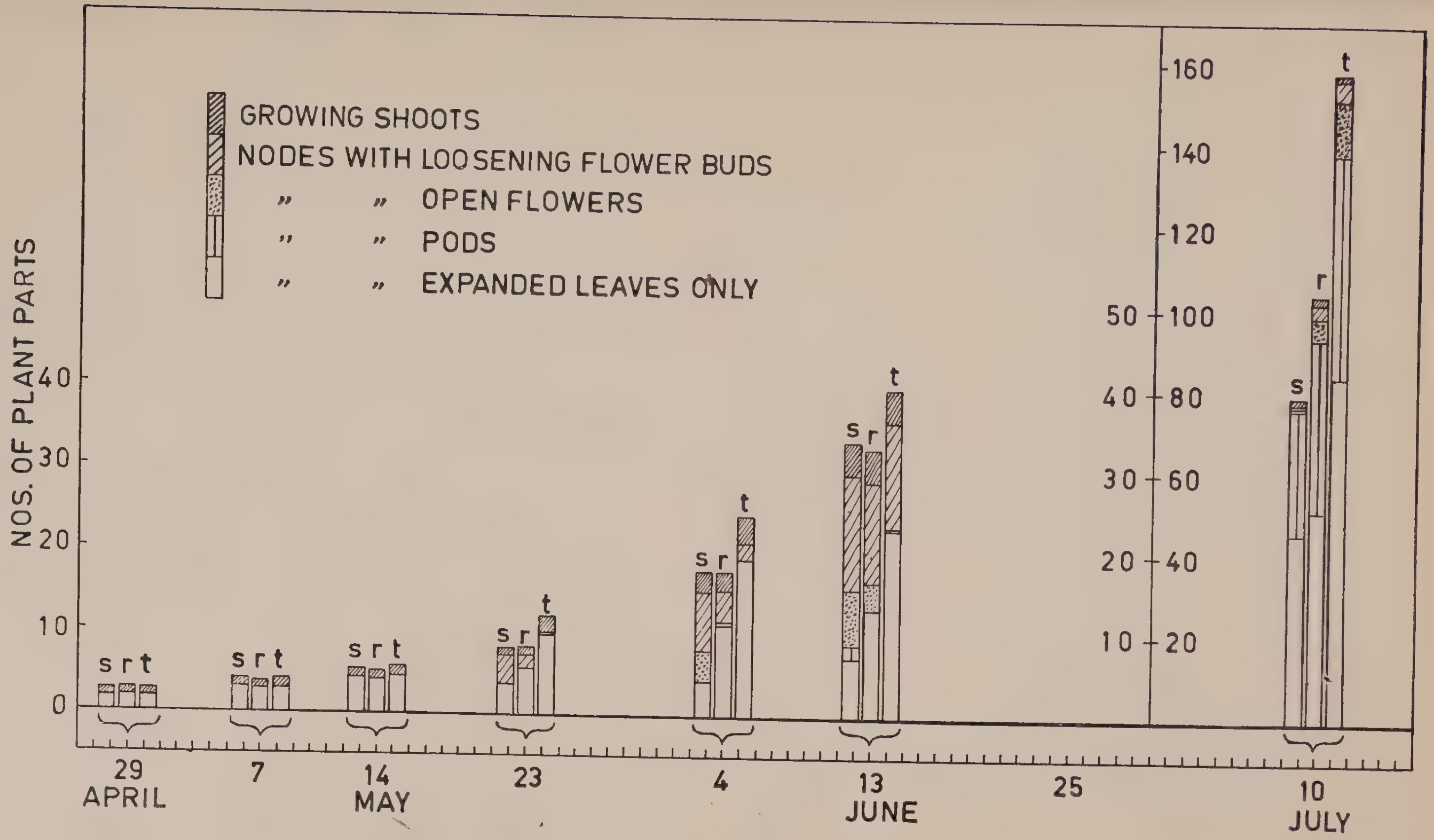

Fig. 1. Growth, flowering and fruiting of Schlanstedt (s), Rastatt (r) and Tick Bean (t). Mean numbers of the various plant parts developed per plant, based on 50 plants of each variety for the first three dates and 20 thereafter.

Through June and July, as the S flowers opened and the pods formed while shoot growth slowed in relation to R, the number of nodes bearing "loose" flower buds on R equalled and then surpassed that on S. Flower-opening and pod-formation on R showed a corresponding initial lag behind S before overtaking. T lagged still more, starting to flower only in mid-June but finally producing more flowers and pods than R.

The later-produced units classed as "growing shoot tips" and "loose" flower buds on any one shoot were naturally less vigorous, containing fewer, smaller and less bunched growing leaves or buds then the earlier ones; later pods were not filled and later flowers fell without opening. That is to say the more numerous leaves and inflorescences produced in late June-July by R, and still more by T, were "starved" by comparison with the organs similarly classified but produced earlier by S. On the other hand the shoot tips of T became the most vigorous in June as the S and then the R slowed down.

TABLE II

Mean yields of pods and seeds.

	Schlanstedt	Rastatt	Tick
No. pods per plant	31.2	46.1	36.4
No. seeds per plant	93.8	123.0	89.1
No. seeds per pod	3.1	2.8	3.3
Wt. seeds per plant, g.	65.5	61.2	46.1
Wt. per seed, g.	0.7	0.5	0.5

Yields (Table II). Filled pods occurred mainly at the several nodes next above the lowest flowering nodes and were derived from flowers that opened in June. In the result, R and not T bore the largest number of pods at harvest in August: significantly more than S ($P < 0.01$) which had produced the fewest organs of all kinds. The R plants also bore the largest number of seeds, but here the differences were smaller because the average R pod contained fewer seeds than the S and T pods. Moreover the individual seeds of R and T were both considerably smaller than the S seeds ($P < 0.01$), so that the total weight of seeds per plant was largest on S, smaller on R and smallest on T; these differences were not significant.

Summing up, the growth pattern of Rastatt fell between that of Schlanstedt and the Tick in this trial: R flowered and ripened later than S, but earlier than T. The early vigorous growth of S was more concentrated in fruits, and of T in shoots, than that of R which was divided more equally between fruits and shoots.

Aphid Incidence

Total A. fabae *on the plants.* Counts of alatae were made on a total of 29 days and there were fewer on R than on S on every day except one. There were fewer alatae on R than T, also, except for 5 days in mid-May and 3 days in mid-June; and fewer on T than S on all but two days. The counts are summarized in Fig. 2 where the daily percentages of all alatae found on the three varieties are shown as means for each period between sprayings. When the alatae began to arrive at the end of April and beginning of May there were an average of 3—4 on S for every one on R or T, but this ratio fell steadily thereafter and was only about 4 : 3 after mid-June. Accordingly, the differences observed on single days between S on the one hand and R or T on the other, were statistically significant on most days in May but not in June. The progeny counts (Table III) show a similar fading of the previously clear varietal differences, by late June when all the plants were maturing.

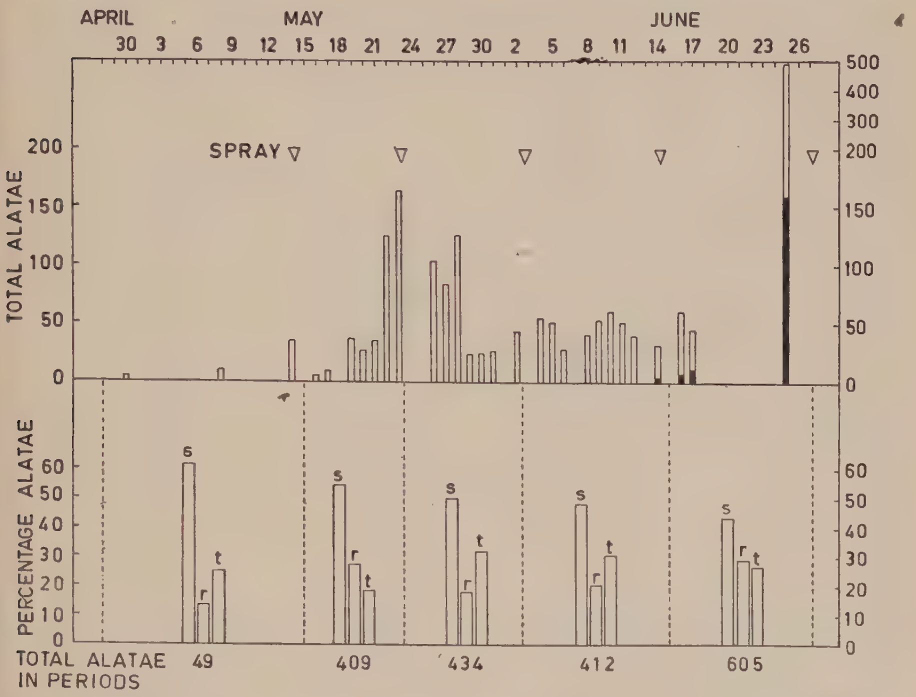

Fig. 2. Alate aphids counted on the plants. Above: combined totals on all plants on individual days. Open columns — *Aphis fabae*; solid columns — *Acyrthosiphon pisum*. Below: mean daily percentages of *A. fabae* on each variety in five successive periods.

TABLE III

Total A. fabae *progeny on the three varieties.*

Date	Schlanstedt		Rastatt		Tick	
	No.	%	No.	%	No.	%
14/5	287	41.3	101	14.4	306	44.2
23/5	451	58.3	135	17.6	184	23.9
25/6	2523	33.2	2155	28.2	2931	38.6

Among plants of one variety there was no correlation between plant height and the number of alatae collected; but there was an "edge-effect" in that the plants along the more exposed north and south borders of the plot collected more alatae than the others.

Distribution of A. fabae *on the plants.* Vigorously growing organs were preferred as they became accessible on all the varieties: the furled leaves at the shoot tips and the loosening flower buds below. Most settled alatae had crawled into crevices but the exposed ones too were commoner on growing shoot tip and flower buds than on expanded leaves, flowers, pods or stem. The progeny were still more concentrated into crevices feeding particularly on bud bases, pedicels and

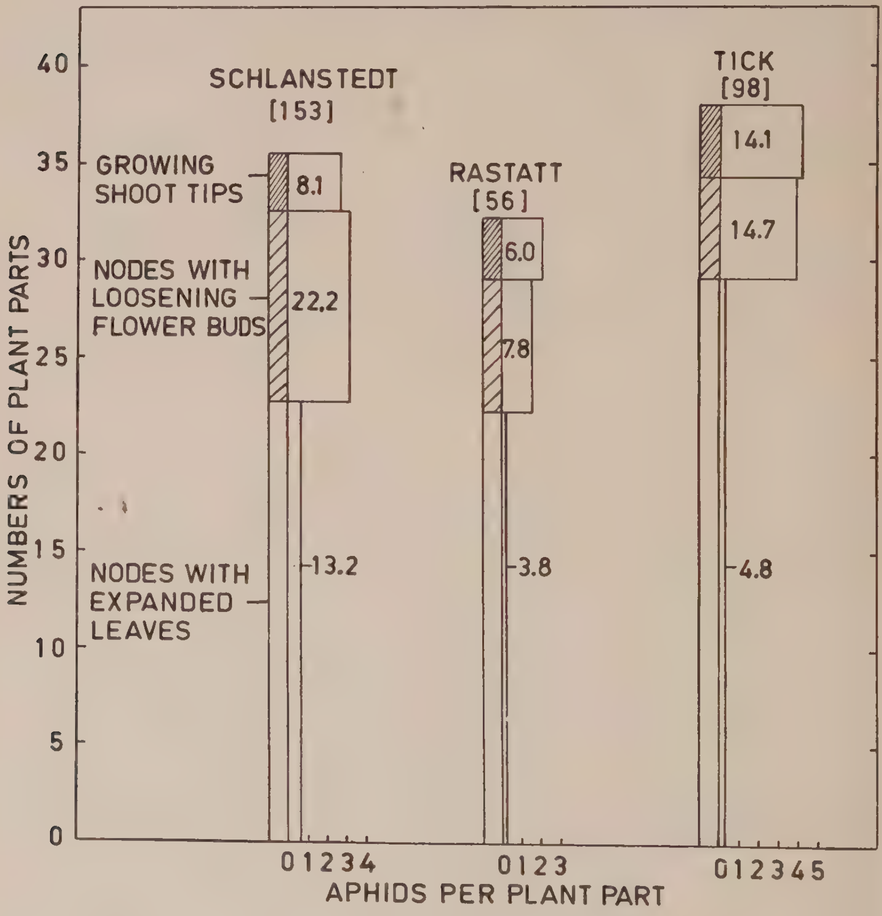

Fig. 3. Distribution of alate *A. fabae* on the plants, June 5—12 (seven counts). Mean numbers of plant parts available through this period shown in the vertical columns. Mean daily percentage aphid incidence among parts and varieties shown by the areas of the blocks against the right side of the columns, the width of each block indicating the relative density of aphids on the parts. Numbers in brackets under varietal names are total aphids for the period.

the adjacent stem. Thus on each variety the aphids were first concentrated at the shoot tips, then in later counts an increasing proportion appeared among the flower buds, and finally they were found more or less evenly distributed up the plant as it matured. Because S flowered first, then R, and then T, these distribution changes occurred 'out of phase' in that order. The drop in the total number of alatae (Fig. 2) and progeny (Table III) on T relative to S and R in May was clearly due to the loosening and colonisation of the flower buds on S and then R, before T (Fig. 1). The proportion of alatae on the flower buds rose to a higher level, and remained high for longer, on S than on R, before it fell again with approaching maturity; on T the proportion on the flower buds never rose much higher than that on the shoot tips.

Table IV and Figs. 3 and 4 illustrate these differences. For the construction of Fig. 3 the numbers of plant parts present on each separate day were estimated by interpolation between the counts made on June 4 and 13 (Fig. 1) to obtain more accurate estimates of aphid numbers per part (densities) which provide the most direct measure of the aphid preferences. Later in June the number of loosening flower buds declined and the records for that period are inadequate to estimate the numbers of plant parts available for colonisation. Fig. 4 therefore shows simply the percentages of alatae on the three classes of plant part.

Early in June the alatae were about three times denser (horizontal axes in Fig. 3) on the shoot tips, and on the flower buds, than on the expanded leaves ($P < 0.001$), and the three varieties did not differ significantly in this respect. But the mean density of alatae on all parts combined was about 40% higher on S and T than on R ($P < 0.01$). The smaller *total* number of alatae (Table IV, and areas in Fig. 3) on R than on S was mainly due to the smaller number settling among the loosening flower buds of R ($P < 0.001$). This in turn was due partly to the lesser density of alatae on the individual R buds, and partly to the fact that at this time there still were fewer nodes with such buds available on R than on S (vertical axis in Fig. 3). The shoot tips of R were rather more numerous (if less vigorous), so that the alatae here, although less dense, were in all hardly fewer than on S. The total number of alatae on the expanded leaves was significantly lower on R than S ($P < 0.01$).

TABLE IV

Mean daily numbers of alate A. fabae *(expressed as square roots) on the shoot tips, flower buds and expanded leaves of the three varieties on seven days June 5—12, and four days June 14—25.*

	June 5—12			June 14—25		
	S	R	T	S	R	T
Shoot tips	1.9	1.6	2.3	4.1	2.5	3.3
Flower buds	3.5	1.9	2.6	3.9	4.3	3.8
Expanded leaves	2.4	1.3	1.4	4.0	3.2	2.7
	Least sig. diffce. at 5% = 0.7 1% = 0.9 0.1% = 1.2			L. s. d. at 5% = 0.9		

The difference between the total numbers of alatae on R and T was not made up in the same way, for in this case the shoot tips contributed as much to the difference as did the flower buds ($P < 0.05$ for both), and the expanded leaves contributed nothing. The higher density of alatae on the flower buds of T more than compensated for the larger number of such buds on the R plants at this time. Earlier, in May and the first days of June, when there were few flower buds on T these naturally contributed less to the difference in total numbers; the shoot tips then carried the majority of alatae on T and were almost entirely responsible for the overall difference between T and R.

The situation changed considerably after mid-June (Fig. 4 and Table IV). Some of the alatae were now found on the pedicels of open flowers and these have been counted in with those on expanded leaves. On the expanded leaves themselves, some preference now appeared for the lowest which were showing signs of incipient senescence. With leaf and inflorescence growth coming to an end on S, the alatae were no longer most numerous on flower buds. But the R plants were still producing relatively vigorous flower buds and now entered the phase when these buds carried more alatae than the other parts. The R buds were now collecting as many alatae as those of S; although the other parts of S were still collecting more and in all S bore some 20% more alatae than R or T ($P <$ 0.05). There was little change in the distribution of alatae among the parts of T, but in all the T plants were no longer collecting more than R.

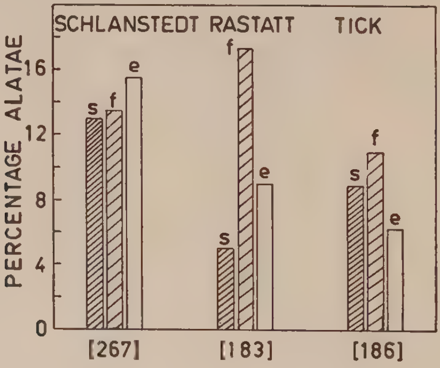

Fig. 4. Distribution of alate *A. fabae* on the plants on June 14—25 (four counts). Mean daily percentage incidence of aphids among parts and varieties with percentages on growing shoot tips (s), nodes with loosening flower buds (f) and nodes with expanded leaves (e) shaded to correspond with Fig. 3. Numbers in brackets are total aphids for the period.

Acyrthosiphon pisum (Harris) behaved very differently from *A. fabae*. First, it did not appear in any numbers until the second half of June (Fig. 2), migration to summer hosts being later in *A. pisum* which overwinters on perennial herbs (Dunn & Wright, 1955). Secondly, although *A. pisum* also colonized shoot tips and flower buds preferentially this was less marked than in *A. fabae* on the same plants at the same time: many more of the *pisum* alatae and progeny were found on expanded leaves. Thirdly, on the days when the *pisum* alatae were counted (June 14, 16, 17 and 25) their numbers differed markedly between varieties although those of *fabae* no longer did. Fourthly, *pisum* consistently preferred Rastatt to Schlanstedt, with Tick intermediate: the opposite of the previous order for *fabae.* An analysis of variance was made on the counts of June 25 when many specimens of both aphid species were present. Of the 159 *pisum* alatae on all the plants, the percentages were 16 on S, 49 on R and 35 on T; R and T both having significantly more than S ($P < 0.01$). Of the 499 *fabae* alatae the percentages were 42, 28 and 30 respectively, with no significant differences. There were no significant varietal differences in the numbers of progeny of either aphid species, the percentages on S, R and T being 21, 53 and 26 respectively for *pisum* (total 295), and 33, 28 and 39 for *fabae* (Table III).

DISCUSSION

The results confirmed MÜLLER's (1958 et ante) finding that *A. fabae* alatae prefer Schlanstedt to Rastatt, but failed to confirm his statement that this is independent of the physiological stage of the plants. The preference was most marked when the plants were young and diminished progressively as growth slowed and maturity approached (cp. MÜLLER, 1953, Fig. 10). The alatae settled and reproduced preferentially on the young vigorously growing organs (i.e. those destined to grow most afterwards) of all three varieties: the shoot tips and especially the young inflorescences. Among these, they preferred more vigorous to less vigorous ones. Thus their preference for the earlier flower buds (as indicated by their density on them compared with other organs on the same plant) was strongest on Schlanstedt which produced the fewest and individually most vigorous ones, and weakest on Tick which produced the greatest number and individually least vigorous ones. The relative incidence of alatae among organs and varieties varied continuously according to the changing availability of vigorously growing organs, which was in turn governed by the different developmental patterns of the varieties. The overall resistance of Rastatt was associated with its "compromise" type of developmental pattern, in which shoot and fruit growth were more evenly balanced (growth less concentrated in either one) than in the other two varieties during the critical weeks of May and early June. The growth of the earliest variety, Schlanstedt, was more vigorous than that of Rastatt, for a short time in the shoots, and more especially in the inflorescences, and it was these two types of organ in the same sequence and timing which collected most of the alatae making up the larger total numbers of Schlanstedt in those weeks. The growth of the latest variety, Tick, on the other hand, was more vigorous in the shoots (leaf-production) than that of Rastatt through those weeks, and these organs collected more alatae than the shoot tips and flower buds of Rastatt combined. When the Tick inflorescences eventually developed Rastatt was producing its later weaker ones, and here too the Tick now collected more alatae. Comparison of Schlanstedt and Tick completes in the circle. The growth pattern of the Tick differed from Schlanstedt's in the same way as did Rastatt's, but more so, since the Tick was still later to flower with individually still weaker inflorescences at their best. Correspondingly, the difference of susceptibility between Schlanstedt and Tick was, to an even greater extent than that between Schlanstedt and Rastatt, due to more alatae being collected by the Schlanstedt inflorescences, the shoot tips contributing nothing.

It may be postulated from the work of LINDEMANN (1948) and MITTLER (1958) that the phloem sap supplying young vigorously growing organs is richer in organic nitrogen than that elsewhere in the same plants. As between plants of different varieties but comparable overall vigour, giving rather similar yields as the three varieties did in this trial, the quality of sap obtainable at the best feeding sites on each is likely to depend on local growth vigour and thus on how much growth is going on elsewhere on the same plant at the same time. Pending direct comparison of sap composition in these varieties, and extension of the work to other varieties, it is therefore suggested that the sap available for aphids on Rastatt was less nutritious than on the other two varieties throughout the critical period of active growth from April to mid-June. Since Schlanstedt filled its pods with fewer leaves but yielded as much as Rastatt, the sap passing out through the phloem of the expanded leaves may also have been richer in Schlanstedt than Rastatt; and more alatae settled on these leaves of Schlanstedt. Very few alatae settled on the expanded leaves of the Tick Bean which had even more leaves.

MÜLLER (1958) states that alate *A. fabae* which have already fed and larviposited on Schlanstedt and Rastatt, leave again sooner from Rastatt (we have confirmed this in several greenhouse experiments), so it is possible that they discriminate sap quality directly. But MÜLLER's (1953, 1958) field observations show clearly that they can also discriminate S from R without feeding or sap-tasting. Their preference for vigorously growing organs which appears to create the preference for S, must therefore be attributed at least partly to responses to superficial plant features (contact chemical stimuli?) and not to the sap itself. Whatever their nature, such stimuli would appear to be "tokens" of sap nutritiousness: the hypothetical "nutrient stimuli" which were distinguished from taxonomic "flavour stimuli" in the dual discrimination theory of KENNEDY & BOOTH (1951). To the circumstantial evidence on which that theory still rests, we would now add the contrasted behaviour of *Acyrthosiphon pisum* on the beans.

Although the results confirm MÜLLER's (1951) finding that *A. pisum* and *A. fabae* showed opposite preferences among the varieties, this was not a reversal of the order of varietal resistance. Rastatt was resistant to *fabae* because it was least favoured by *fabae* during the critical period of active plant growth. There is no evidence on the preferences of *pisum* during that period, nor on varietal resistance to *pisum*, because this aphid did not appear until the plants were maturing. The very fact that *pisum* showed marked varietal preferences when those of *fabae* were disappearing suggests that the plant stimuli which decided the varietal preferences of the two aphids were different. *A. pisum* is confined almost exclusively to Leguminosae (HILLE RIS LAMBERS, 1947). *A. fabae* is notoriously damaging to one very vigorous legume, *Vicia faba*, but colonizes members of many other plant families as well. On its primary host *Euonymus europaeus* L., the plant species to which *A. fabae* is most closely adapted, it behaves like *A. pisum* on beans. It is less confined to growing and senescing organs and spreads more on to mature leaves, than *Myzus persicae* (Sulzer) for which *Euonymus* is not a favoured host (KENNEDY, IBBOTSON & BOOTH, 1950). In both cases the aphid which is more closely adapted to the taxonomic status of the plant is less discriminating with regard to its physiological status; and the same thing is found among different forms of one aphid species (KENNEDY & BOOTH, 1954). Thus the contrasted varietal preferences of *A. pisum* and *A. fabae* on the beans tend to strengthen the conclusion that the resistance of Rastatt to *A. fabae* is physiological, a matter of growth pattern.

It is clear that the deliberate creation of this type of resistance would not be a straightforward task. The selection for vigorous growth and high yields to which all crop plants have been subjected, may well have enriched their sap and increased their susceptibility to aphids automatically. That this unfortunate consequence can be mitigated to a useful degree is suggested by the differences of susceptibility still found among varieties of comparable yield, but different growth pattern. At the same time it implies that a higher-yielding variety than Rastatt may be less resistant to *A. fabae* even if its growth pattern is in some respects similar. Indeed a growth pattern like Rastatt's in one respect, the concurrence of shoot and fruit growth, is seen in the large-seeded high-yielding Windsor or Garden Bean which flowers earlier than the Field Beans (CHAKRAVARTY, DRAYNER & FYFE, 1956) but tillers early as well, and this was the most susceptible variety of all in DAVIDSON's (1922) tests. The possibility of obtaining varieties as resistant as or more so than Rastatt, yet higher-yielding, is not excluded, but it appears that early flowering, with fruiting concentrated in a few pods and large seeds (as in the Garden Bean and Schlanstedt) is particularly to be avoided, although extreme lateness (Tick) may again mean susceptibility. At first sight it might appear that resistance based on growth pattern would be an unreliable type of resistance to aim at in practice. Nevertheless, as mentioned in the Introduction, present indications are that many of the existing cases of useful resistance to aphids in crop plants may be of just this type.

We are indebted to the Norwegian Agricultural Research Council and to the Norwegian Research Council for Science and the Humanities who financed the first author's visits to England, to Professor V. B. WIGGLESWORTH for granting her facilities with the Agricultural Research Council Unit of Insect Physiology, to Mr. C. O. BOOTH for practical assistance, and to Mr. D. V. LINDLEY of the Statistical Laboratory, Cambridge for statistical help.

ZUSAMMENFASSUNG

In einem kleinen Parzellenversuch wurden drei Ackerbohnensorten verglichen: Rastatter (bekannt als relativ resistent gegen *Aphis fabae*), Schlanstedter (sehr anfällig) und eine englische Tick Bohne. Das Wachstum der Pflanzen und der tägliche Befall mit geflügelten *Aphis fabae* und *Acyrthosiphon pisum* wurden aufgezeichnet (protokolliert), dabei hielten periodische Spritzungen mit Aphidiciden die Schädigung der Pflanzen möglichst niedrig.

Schlanstedter blühte und fruchtete als erste, danach Rastatter (intermediär) und Tick mit den meisten Trieben, Blättern und Blüten zuletzt. Die Erträge von Schlanstedter und Rastatter waren annähernd die gleichen, die von Tick etwas niedriger. Der relative Befall mit *Aphis fabae* stand sowohl auf den Organen einer Sorte wie zwischen den Sorten in

Beziehung zur Wuchsform. Die geringere Anzahl der Blattläuse auf Rastatter im Vergleich sowohl mit Schlanstedter wie mit Tick war mit dem „Kompromiß"-Wachstum der Rastatter verbunden, wobei das Wachstum während der kritischen Wochen zwischen Trieben und Früchten gleichmäßiger verteilt und offensichtlich bei den einzelnen Organen weniger kräftig ist.

Zusammen mit dem gegensätzlichen Verhalten von *A. pisum* und früheren Arbeiten über die Phloemsaftzusammensetzung in Beziehung zu Pflanzenwuchs und Aphidenernährung weisen diese Beobachtungen darauf hin, daß der Phloemsaft für die Blattläuse bei der Rastatter am wenigsten nahrhaft sein dürfte und daß die Resistenz der Rastatter gegen *Aphis fabae* auf der Reaktion der geflügelten Migranten auf einige oberflächliche Pflanzeneigenschaften beruhen könnte, die mit Wachstumseigentümlichkeiten und der Saftzusammensetzung verbunden sind. Mögliche praktische Folgerungen werden kurz erwähnt.

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RECTIFICATION

Ent. exp. & appl. 1 (2) — 1958 —, page 80 (Publication P. GRISON): Table II : „*Feuilles âgées*" doit placé à droit, „*Feuilles jeunes*" à gauche.

Le texte juste de la Table II est imprimé ci-dessous encore une fois :

Aliment : Ackersegen	Feuilles jeunes		Feuilles âgées	
Prélèvement à	9 h.	17 h.	9 h.	17 h.
en 1949				
sucres totaux	44,0	55,7	55,6	63,6
azote total	53,0	55,0	41,0	47,0
C/N	0,63	0,83	1,10	1,16
indice fécondité	*13,3 oeufs*	*3,6 oeufs*	*2,2 oeufs*	*2,3 oeufs*
en 1953				
sucres totaux	32,0	50,0	25,0	42,6
azote total	61,0	69,0	28,0	33,5
C/N	0,52	0,72	0,89	1,27
indice fécondité	*11 oeufs*	*20 oeufs*	*2 oeufs*	*1 oeuf*
en 1954				
indice fécondité	*26,7 oeufs*	*22,6 oeufs*	*7,8 oeufs*	*8,6 oeufs*
choline totale	0,274		0,079	

Ent. exp. & appl. 1 (1958): 241—245. *North-Holland Publishing Co., Amsterdam*

THE REARING OF CRANE FLIES (TIPULIDAE)

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A simple method for the culture of *Tipula oleracea* L. & *T. paludosa* Meig. is described. Eggs obtained from decapitated fertilised females are incubated on damp filter paper. Larvae are kept in damp, drained sand and fed on powdered dried grass. Pupae are kept in damp blocks of plaster of paris.

The literature on leatherjackets is fairly sparse and no author gives a satisfactory culture method. The culture of soil insects is never easy but the method described in this paper is relatively simple both to set up and to manage. The method has only been used to rear *Tipula paludosa* Meig. & *T. oleracea* L. but may be useful for other Tipulids. An attempt was made to rear 100 larvae of *T. fulvipennis* Degeer but, after growing quite well for about ten weeks, they appeared to stop feeding and by twenty weeks all had died.

Handling of eggs

T. paludosa females will mate and lay eggs within a day of emergence. *T. oleracea* females, though they will mate soon after ecdysis, do not lay eggs until three or four days later. *T. paludosa* females, given favourable conditions for oviposition, will usually start to lay as soon as copulation is finished and will continue until all the mature eggs in the body are laid. *T. oleracea* females, even if ready to lay — i.e. three to four days old, may take up to fourteen days to lay all their eggs.

To obtain eggs of known age it is most convenient to decapitate a mature fertilised female (of either species), cut off wings and legs and 'float' the body on water where it is supported in the surface film. Under these conditions, eggs are usually laid rapidly and without pause until no more are left. For example, a *T. paludosa* female laid 971 eggs in two and a half hours, about six eggs per minute. At the end of this time there were only nine mature eggs left in the body. Rates of up to fifteen eggs a minute have been noted.

The ovipositing female is 'floated' on water for two reasons. The first is that an ovipositor working in air flicks eggs out with considerable force. They may land several inches away. In water they are trapped and sink to the bottom. If a piece of filter paper is put under the water the eggs settle on this. When the female has laid all her eggs the water is drawn off and the eggs lifted out on the damp filter paper, ready for incubation. The second reason is that eggs of both species are very susceptible to desiccation in the first few minutes after laying. In dry air they take only two to four minutes to collapse. This susceptibility is lost very quickly and later in the incubation period the egg may take up to 90 minutes to collapse. For the first few critical minutes after the egg is laid, therefore, it is essential that it be kept wet.

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*) Agricultural Research Council, Unit of Insect Physiology.

The eggs are incubated on damp filter paper. At 21° C, *T. oleracea* eggs take six days, *T. paludosa* eggs ten days to hatch.

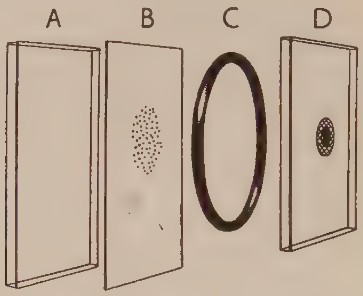

Fig. 1. Exploded drawing of egg container.

A — Perspex back, 3″ × 2½″ × ⅛″
B — Filter paper, 3″ × 2½″, bearing eggs
C — Synthetic rubber ring, 2″ diameter
D — Perspex plate, 2½″ × 2½″ ×⅛″, with gauze covered ¼″ hole

The container shown in Figure 1 was developed to satisfy three conditions: 1. that there should be no small cracks or holes through which the active, newly hatched larvae could escape; 2. that the filter paper should be kept constantly moist but no wet in the vicinity of the eggs; 3. that the egg container should be ventilated.

The two transparent plates are of perspex and the washer holding them apart is of synthetic rubber. The front plate is drilled and a circle of 100 mesh phosphor-bronze gauze pressed into the perspex with a hot (about 150° C) iron. The plates, washer and filter paper bearing the eggs are held together with an elastic band and the container stands in ¼″ of water.

Condition 3 is necessary for two reasons. A container with no air hole usually acquires a pool of water which makes the filter paper too wet for the hatching larvae if not for the eggs. Even where no pool of water develops, batches of eggs in totally enclosed containers have often failed to hatch when eggs from the same female have hatched successfully in an aerated chamber.

There can be considerable variation in duration of incubation period between the eggs of any one batch. The variation is of a peculiar kind however since the majority of eggs hatch on the sixth and tenth days (*oleracea* & *paludosa* respectively) with just a few eggs hatching every day thereafter for three or four days. Examination of unhatched eggs remaining after the hatching peak shows mainly live full term embryos. It seems likely that the hatching process requires precise and critical conditions and that even in the chambers provided these conditions are not always satisfied.

CULTURE OF LARVAE

The leatherjackets are kept in damp, drained sand and fed on dried powdered grass. This choice of food (known as dried grass meal commercially) was more or less fortuitous. Dried grass samples are used extensively in the Agricultural Chemistry Department in Newcastle and a considerable quantity of surplus material was available. It is a high protein food, keeps indefinitely and, when wetted for use in the culture, is friable and pleasant to handle.

A number of other foods was tried but when dried grass became available and proved successful no further search was made.

The sand used is a fine sea sand which passes through a 25 mesh and is retained by a 100 mesh sieve. The sand is thoroughly washed before use and thereafter is used over and over again. Repeated use of the same sand necessitates a cleaning technique which is described below.

This sand will hold up to 24% of water by weight. If a watertight container is to be used then the water and sand must be measured to give a water content of not more than 20% (for this grade of sand). It is simpler to use a container with a gauze covered hole in the bottom through which excess water can drain off.

Circular boxes (six inch diameter, two inch high) have been made out of rigid polyvinylchloride (P.V.C.). In lid and base are holes covered with 100 mesh phosphor bronze gauze. The metal gauze is pressed into the plastic with a hot iron. The lids must fit well to prevent the youngest larvae escaping. Plastic is recommended since it is unaffected by prolonged contact with wet sand. The gauze-covered hole in the lid appears to be necessary to keep the powdered grass sweet. If air is excluded the wet grass ferments and the culture turns sour.

The six inch diameter boxes are used for stock cultures. A number of larvae are kept in each box (see p. 245). For the culture of individual leatherjackets, short (one and a half inch) lengths of ⅞ inch diameter glass tubing are used. Both ends are capped with 100 mesh gauze discs welded to polythene collars fitting tightly over the glass tubing.

To start a culture, a six inch box is filled with dry sand to a depth of one and a half inch and allowed to soak up water through the base. The sand surface is dusted lightly and unevenly with dry powdered grass with a few small heaps of grass here and there. The filter paper bearing hatching eggs (see previous section) is laid on the sand and is also dusted with grass. The lid is put on and the box can be left for three to four weeks. It should be opened at least once a week, powdered grass and water being added as necessary. Water is rarely necessary for the sand stays quite moist for at least a fortnight even on the bench. Once the culture is started the powdered grass is wetted and made into a paste before putting in the box. It should be put down in lumps rather than spread evenly over the surface.

After three to four weeks at 20° C the larvae of both species are big enough to see and handle comfortably. The pot must be cleaned out and the larvae transferred to clean damp sand with lumps of fresh grass paste available. It is advisable to score the sand surface with a sharp point before putting the larvae in. If the sand is smooth they cannot dig tunnels in the sand and stay crowded on the surface with greater risk of fatal encounters with other larvae.

After this first cleaning the larvae should be inspected and fed each week and cleaned out every fortnight. After about seven weeks (*T. oleracea*) and fourteen to fifteen weeks (*T. paludosa*) at 20°.C pupae should start to appear. The boxes should then be inspected every day and visible pupae removed. The relatively helpless pupa is very liable to be killed and eaten by the larvae remaining in the box (especially in cultures of *T. oleracea*). This mortality can be minimised by reducing the number of older larvae in each box. The only way to eliminate the risk is to keep the older larvae in individual tubes as described above; for a stock culture, the extra work is usually not worth the saving in mortality.

Cleaning Technique. Repeated use of the same sand and containers soon leads to fungus infestations. The metal gauze becomes clogged and the sand becomes waterlogged. The sand eventually becomes unwettable.

The remedy for this is to soak the gauze for one to two hours and the sand overnight in a solution of domestic bleaching agent containing 0.03% of available chlorine. 0.3% of detergent (Teepol) is added to the solution.

After this treatment the gauze is washed under a strong jet of water and the sand is washed until the water no longer froths; the Teepol, as well as facilitating the action of the bleaching agent acts as an 'indicator' for the completion of the washing process.

HANDLING OF PUPAE

Pupae are kept in holes ($^5/_{16}$″ diam., $^3/_4$″ deep) in damp blocks of plaster of paris. The block is kept in a bag of thin polythene film to retain moisture and contain the emerging flies.

A culture of *T. oleracea* larvae, even if all the larvae are of the same age, may take a month to pupate at 20° C. In *T. paludosa* cultures, also at 20° C, the first pupa may appear as much as two months before the last. If the culture is a small one, it is likely that there will be very few males left alive to fertilise the females (males tend to pupate and emerge first).

As the pupae appear they are put into the plaster blocks and kept at a temperature of about 5° C. This treatment slows up the development of the pupae as they appear in the culture and it is usually possible to hold up emergence until the whole culture has pupated. The pupae are then returned to 20° C and the adults emerge over a period of, at most, ten days. The treatment does not seem to increase the mortality of pupae significantly.

If a continuous, 'all-the-year' supply of material is required, cultures of *T. oleracea* can be started at fortnightly intervals (*T. paludosa* at monthly intervals). The cooling treatment for pupae is then unnecessary since the females from one culture emerge about the same time as the males from the next.

MORTALITY

With cultures of both species the general experience has been that mortality is fairly high in the first twenty to thirty days after hatching though largely dependent on the number in the box. However many newly hatched larvae are put in the box, there will be probably about forty and rarely more than 100 left at thirty days. For example, 1085 first instars, put in a six inch diameter box as they hatched produced eighty larvae at thirty days, while in another box seventy-four first instars produced sixty-three larvae at forty days.

After this period of high mortality there is usually very little death until pupae start to appear. Again, mortality is correlated with larval density. Very few larvae are lost from *T. oleracea* cultures having up to thirty larvae per box. There is some indication that *T. paludosa* cultures can be more crowded, perhaps up to sixty or seventy larvae per box with little loss. However, at these densities, mortality will tend to become high as the larvae get bigger so it is not recommended that more than thirty larvae of either species be kept in one 6″ diameter box.

Mortality is also correlated with the frequency of cleaning out the culture. The larger leatherjackets live in tunnels in the sand and, if food is plentiful seem to move about very little. When the larvae are transferred to a fresh box they are active for some time, remaking their tunnels, and their chances of meeting and damaging other larvae are increased. The routine suggested on p. 244 keeps the culture clean with the minimum of disturbance.

When pupae start to appear mortality increases. This is mainly due to cannibalism, the active larvae eating the relatively helpless pupating larvae and pupae. Cannibalism is not the only cause however. Among larvae kept in individual tubes deaths are very rare except at pupation time when a few may occur, particularly in *T. paludosa.*

ZUSAMMENFASSUNG

1) Befruchteten weiblichen Schnaken werden die Köpfe, Flügel und Beine entfernt, um sie zur Eiablage zu veranlassen. Der Körper „schwimmt" auf der Wasseroberfläche einer Schale, so dass man die Eier auffangen kann.

2) Die Eier werden auf einem Filtrierpapierstreifen ausgebrütet, der unten senkrecht in Wasser steht. Die ausschlüpfenden Larven werden dadurch am Entkommen verhindert, dass man das Filtrierpapier in einen Behälter einspannt, der aus zwei „Perspex"-Platten und einem Gummiring besteht.

3) Man hält die Larven einzeln oder in Gruppen in feuchtem drainiertem Sand. Sie werden mit einem Brei aus gepulvertem, getrocknetem Gras gefüttert.

4) Die Puppen hält man in Löchern eines feuchten Gipsblocks. Sie können kühl gehalten werden, so dass männliche und weibliche Fliegen zusammen ausschlüpfen.

5) Die Hauptursache für die Sterblichkeit ist Kannibalismus. Es werden Möglichkeiten diskutiert, um die Sterblichkeit auf einem Minimum zu halten.

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INFLUENCE DE CERTAINS FACTEURS ECOLOGIQUES SUR LA PONTE ET LE DÉVELOPPEMENT DES CÉTOINES GRISES

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Des élevages de laboratoire ont été entrepris pour préciser l'action des principaux facteurs ecologiques sur le développement des larves de Cétoines grises. Les recherches ont porté surtout sur *Epicometis squalida* Scop. et *Oxythyrea funesta* Poda.

L'influence de la température et de la nature du milieu fut plus particuliérement étudiée. Ces élevages mettent en évidence les différences de comportement selon l'espèce considérée, en ce qui concerne le rôle de la température, la sensibilté aux maladies ou les besoins alimentaires. *O. funesta* est l'espèce la moins exigeante comme conditions de milieu et la plus résistante aux mycoses.

Ces faits expliquent vraisemblablement les différences de répartition géographique de ces coleoptéres: *O. funesta* étant plus largement répandu que les *Epicometis*.

Certaines années, dans certaines localités, des dégâts sont occasionnés au printemps aux fleurs des arbres fruitiers par de petits colèoptéres: les Cétoines grises. Ces insects, ainsi appellés à cause de l'abondante pilosité grise recouvrant leur corps noir tacheté de blanc, ont été signalés comme nuisibles à plusieurs reprises par divers auteurs. Ces dernières années, leurs ravages ont été notés en 1949 dans la région de Marrakech (Maroc) par Perret, sur agrumes; en 1948 par J. B. Dental sur plantes horticoles et arbres fruitiers sur la Côte d'Azur; par P. Schmerber en Alsace sur fleurs de Pommiers, mais aussi de Colza, culture sur laquelle elles se sont révélées très dangereuses; par P. Bovey en Suisse sur Pommiers et Prairies. Cette année là parait avoir été une période de pullulation des Cétoines grises en différents points de leur aire d'extension, depuis le Maroc jusqu'à l'Alsace.

La biologie de ces insectes qui appartiennent à trois espèces distinctes: *Epicometis hirta* Poda, *Epicometis squalida* Scop. et *Oxythyrea funesta* Poda a fait l'objet d'observations détaillées de plusieurs auteurs et en particulier de Bebic (1954), de Bohm (1950) et de Guennelon (1958) -Celle-ci a étudié un foyer de Cétoines grises dans la région d'Avignon depuis 1953.

D'après ces publications, les insectes parfaits, floricoles, volent au printemps aux heures chaudes et ensoleillées de la journée, ils se nourrissent des fleurs d'une grande quantité d'espèces végétales. Les larves ont un développement relativement rapide car les imagos de la nouvelle génération sont formés dès l'automne suivant, ce sont eux qui hivernent, enfouis dans le sol mais ils peuvent occasionellement être actifs par les belles journées de Septembre ou Octobre. Le cycle est donc annuel. En fait bien des points méritaient d'être précisés dans ce cycle évolutif. En particulier, les lieux de ponte sont très mal définis, la durée et les modalités de la croissance larvaire sont très mal connues et l'influence des principaux facteurs du milieu reste à déterminer pour tous les stades: oeufs, larves, imagos.

A l'occasion de l'étude comparative de l'ecologie des principales espèces de Scarabeides phytophages que nous avons entreprise depuis plusieurs années, nous avons cherché a combler ces lacunes en complétant les faits d'observation indiqués par les auteurs par une expérimentation en laboratoire.

Par l'intermédiaire d'élevages en laboratoire, nous avons essayé de mettre en évidence, le rôle des principaux facteurs ecologiques: température, nature du milieu, sur le développement des oeufs et des larves.

Dans des élevages préliminaires, il est apparu que certaines affections dues à des champignons, ou mycoses, provoquaient une mortalité gênante pour l'interpretation des expériences. Il est vraisemblable que la prolifération des champignons est due, en grande partie, à la nature humifère du milieu utilisé: terreau ou débris végétaux en décomposition. Cependant, les maladies dans le cas des Cétoines grises, ne prennent jamais le caractère d'épizooties que nous avons noté chez d'autres Scarabeides saprophages tels que *Cetonia aurata* ou *Oryctes nasicornis* dans les mêmes milieux et les mêmes conditions d'élevage. Il y a donc une sensibilité spécifique de chacun de ces coleoptères vis-à-vis des mycoses.

Ce sont toutes ces questions que nous nous sommes proposés d'examiner en plaçant en élevage des larves d'âge connu, dans des conditions déterminées de milieu. Pour avoir des oeufs, puis de jeunes larves prélevées au moment de l'éclosion, il était necessaire d'obtenir la ponte des imagos dans nos cages. Nous donnerons donc d'abord, la technique d'élevage dont nous nous sommes servis pour les trois espèces de Cétoines grises vivant en France.

REMARQUES SUR L'ELEVAGE DES INSECTES PARFAITS

Nos élevages de larves ont été effectués en partant d'oeufs déposés en cages par les imagos qui nous étaient expédiés des lieux de récolte dans le Midi de la France 1).

Ces insectes étaient mis généralement, au laboratoire, par lots de 100 à 200 dans des cages grillagées de 60 cm × 25 cm × 25 cm dont le fond était garni de terre sur 10 cm environ d'épaisseur. L' alimentation était assurée par un bouquet de fleurs diverses, surtout fleurs de *Heracleum spondylium* et de *Taraxacum dens-leonis*. Dans certains cas, nous avons fait des élevages par couples en verre de lampe tempête disposés sur un pot à fleurs plein de terre.

Les oeufs, qui sont pondus isolément dans une petite cavité ménagée dans une boule de terre, étaient dénombrés une ou plusieurs fois par semaine. La ponte est échelonnée sur plusieurs jours et d'autre part, les premières sorties de terre après l'hivernage ont lieu également pendant toute une période qui reste à déterminer. Aussi les insectes utilisés, qui provenaient de récoltes dans la nature avaient le plus souvent déposé une partie plus ou moins importante de leurs oeufs avant leur capture. Par suite, le nombre d'oeufs observés dans nos cages ne traduit

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1) Outre Mme GUENNELON, de la Station de Zoologie Agricole du Sudest, qui nous a fourni plusieurs milliers d'insectes ramassés aux environs d' Avignon, nous tenons à remercier MM. COUTIN et DUSAUSSOY, de la Station Centrale de Zoologie, qui nous ont fait parvenir des *O. funesta* récoltés dans les Cévennes.

pas la valeur exacte de la fécondité. Pour *E. squalida*, par exemple, dans les élevages en groupe: 275 femelles ramassées le Ier Juin 1954 fournirent 1160 oeufs, ce qui représente 4 oeufs en moyenne par individu; 142 femelles du 17 Mai 1956 donnérent 852 oeufs soit en moyenne 6 par femelle, et en 1957, 67 femelles du 6 Mai et 20 femelles du 17 mai pondirent respectivement 463 et 144 oeufs (7 par insecte dans chaque cas). En fait, d'après les dissection des femelles mûres et les pontes obtenues en élevage isolés, le nombre moyen d'oeufs d'une femelle est voisin de 25 pour les trois espèces de Cétoines grises.

Beaucoup d'insectes meurent après la ponte, mais il y en a qui sont capables de mûrir une seconde série d'ovocytes et d'effectuer une seconde ponte, dont nous ne pouvons fixer l'importance, faute d'éléments en nombre suffisant. Cette seconde ponte a été observée à quelques reprises dans nos élevages bien que l'échelonnement des dépôts d'oeufs rende le phénomène beaucoup moins net que chez le Hanneton, par exemple. La présence d'oeufs mûrs ou en formation dans les ovarioles ayant déjà un „corps jaune" (qui est coloré en verdâtre chez ces Coléoptères) bien visible, confirme l'existence de cette deuxiéme ponte. Il est possible que des troisièmes pontes se produisent dans la nature, mais cette hypothèse reste à vérifier.

En élevage, à 22° C environ, la longévité des insectes dépend naturellement de leur date de récolte: plus celle-ci a été tardive moins les Cétoines peuvent être conservées longtemps, comme l'indiquent les chiffres du tableau I, qui se rapportent dans chaque cas à une vingtaine de couples.

TABLEAU I

Longévité en élevage, à 22° C. des Cétoines grises.

Date de la récolte	Longévité des mâles			Longévité des femelles		
	Max.	Min.	Moy.	Max.	Min.	Moy.
Epicometis squalida						
12 Avril	60 j.	10	39 j.	60 j.	17	46 j.
29 Mai	23	10	17	23	16	18
21 Juin	14	7	8	14	7	10
Epicometis hirta						
12 Avril	48 j.	10	30 j.	48 j.	10	30 j.
3 Mai	41	14	22	39	14	23

Il n'y a pas de différence significative entre les sexes, et la vie dans nos élevages apparaît plus brève que dans la nature.

Le developpement de l'oeuf

A la ponte l'oeuf est un ellipsoïde blanc pur. En se développant il change à la fois de forme et de couleur: il devient sphérique et se teinte de brun. Les modifications sont dues à la formation de l'embryon et à l'absorption d'eau suivant un processus analogue à celui que nous avons étudié chez *M. melolontha* (Hurpin, 1956) et qui est fréquent chez les insectes; il en résulte également une augmentation de volume et de poids. Pour *Epicometis squalida,* l'oeuf qui vient d'être pondu pèse 2 mg., peu de temps avant l'éclosion il atteint 5 à 6 mg.

Action de la température

Nos essais ont été effectués dans de petites boites en aluminium contenant, dans de la terre suffisamment humide, 20 oeufs âgés au plus de quelques jours, prélevés dans les cages d'élevage décrites au paragraphe précédent.

Ces récipients furent placés en polythermostat aux diverses températures constantes, à 1° C. près, mentionnées dans le tableau II, en ce qui concerne *Ep. squalida.*

D'après ces données la température optimum pour l'incubation des oeufs, définie par le maximum d'éclosion dans les meilleurs délais, est de 23° C, chiffre qui est le même qui celui que BEBIC (1954) signale pour *E. hirta.*

TABLEAU II

Influence de la température sur le développement des oeufs de Epicometis squalida.

Température	Nbre d'Oeufs	Nbre de larves	% éclosion	Durée en jours		
				Max.	Min.	Moy.
11°	48	0		après 100 jours		
	100	0		après plus de 4 mois		
15°	120	89	74	42	37	40
18°	120	96	80	21	16	18
20°	132	56	42	20	15	16
23°	120	77	64	16	9	12
25°	132	69	52	13	9	11
27°	112	36	32	12	7	9
30°	100	62	62	9	6	7
34°	70	5	7	—	6	—

Les oeufs de la Cétoine étudiée paraissent moins exigeants au point de vue thermique que ceux du Hanneton commun, il n'y a guère de différences dans l'importance de la mortalité de 15° à 30°. Au-dessus de 30°, les éclosions deviennent rapidement moins nombreuses et 34° semble la limite supérieure pour le développement des oeufs. A 11° les oeufs n'évoluent que très lentement et ils ne parviennent pas au terme de la vie embryonnaire, même après plusieurs mois.

Quelques essais faits selon la même méthode à 10-20 et 30° sur des oeufs de *O. funesta* montrent que les exigences thermiques pour le développement embryonnaire de cette espèce sont très voisines de celles de *E. squalida.* A 10° il n'y a pas d'éclosion, à 20° elles interviennent au bout de 15 jours en moyenne, contre 8 jours environs à 30°.

LA VIE LARVAIRE

Nos recherches sur les larves ont été réalisées en les élevant dès leur sortie de l'oeuf, dans des boites en aluminium garnies de matériaux variés à diverses températures. Nous avons pu ainsi suivre les différentes phases de la vie larvaire, préciser le nombre et les modalités des mues, les conditions d'apparition des maladies et mettre en évidence l'influence de la température ambiante et de la nature du substrat sur la mortalité et la croissance des larves des trois espèces de Cétoines grises.

Etude des mues

On compte trois stades larvaires et deux mues. L'examen des capsules céphaliques (tableau III) et l'observation de larves non pigmentées venant de subir une mue, le prouvent.

Pour *Epicometis squalida*, la premiére mue se produit quand les insectes pèsent en moyenne 40 mg (± 10 mg), la deuxième mue a lieu à 150 mg (± 25 mg) et la nymphose intervient quand les larves atteignent environ 500 mg, mais les différences individuelles s'accusent à ce moment et nous avons enregistré quelques jours avant la prénymphose des larves pesant de 300 à 700 mg.

TABLEAU III

Largeur des capsules céphaliques des différents stades larvaires de Cétoines grises

Stade	*Epicometis squalida*			*Oxythyrea funesta*		
	Max.	Min.	Moy.	Max.	Min.	Moy.
Premier (L1)	1,6	1,4	1,5	1,0	0,9	0,95
Deuxiéme (L2)	2,4	2,1	2,2	1,7	1,4	1,5
Troisiéme (L3)	3,3	2,9	3,1	2,6	2,2	2,4

Ces chiffres, en millimétres, ont été obtenus en mesurant au micrométre oculaire sous la loupe binoculaire la plus grande largeur des capsules céphaliques au niveau de l'insertion des antennes, sur 30 à 50 insectes dans chaque cas.

Pour *Oxythyrea funesta*, les chiffres sont: 15 mg (± 3 mg) pour la premiére mue, 90 mg (± 15 mg) pour la deuxième et environ 400 mg pour la nymphose.

A la fin de son évolution, la larve s'enferme dans une coque construite en agglomérant des débris végétaux et des excrétas avec le liquide évacué de la poche rectale.

Au moment de sa confection, les mouvements de la larve à l'intérieur de cette coque totalement close rendent sa surface interne bien lisse.

La larve ne s'alimente plus et elle vide son tube digestif postérieur au cours de l'édification de la coque. C'est une prénymphe qui présente l'aspect caractéristique de ce „pseudostade" chez les Scarabéides: le corps est devenu quasi rectiligne, les pattes ne sont plus fonctionnelles, le pygidium est de la même couleur blanc-ocre que le reste de la larve. Cette période de la vie larvaire correspond au début de la métamorphose: les processus d'histolyse commencent et d'autre part le tégument nymphal s'élabore.

A cause de la coque il est difficile de suivre les différentes phases de la métamorphose chez les Cétonides: il faut ménager une petite ouverture permettant de voir l'insecte. En opérant ainsi, on perturbe l'atmosphère de l'intérieur de la coque. Cependant des essais effectués avec *Cetonia aurata*, espèce qui fabrique également des coques, montrent qu'il n'y a pas de différence sensible entre la mortalité enregistrée dans des coques ouvertes et celle qui intervient dans des coques laissées intactes.

Nous avons vérifié qu'il en est de même pour les Cétoines grises qui nous étudions ici. Les prénymphes de *E. squalida* et de *E. hirta* pèsent de 150 à 250 mg, celles de *O. funesta* de 150 à 200 mg. Pour les nymphes les poids sont respectivement de 150 mg (± 50 mg) et de 175 mg (± 25 mg). Il y a donc moins de différences pondérales entre les nymphes et, par suite, les imagos, de ces deux espèces qu'entre leurs larves. Les larves d'*E. squalida* se „vident'' relativement plus au moment de la prénymphose.

Influence de la température

Pour toute espèce d'insectes, la détermination de la température optimum de développement est une des premiéres questions à examiner pour la mise au point d'une technique d'élevage.

Au cours de nos premiers essais d'élevage, en 1954, nous avons placé nos récipients au laboratoire à 20—22° C. Il en est résulté une forte mortalité. Aussi, nous avons cherché à estimer de façon aussi précise que possible l'action de diverses températures maintenues constantes sur la vitesse de croissance et l'importance de la mortalité des larves de *Epicometis squalida* et de *Oxythyrea funesta.*

a) *Larves de* E. squalida

Dans une premiére série d'essais, réalisée en 1954, nous avons disposé, en polythermostat, des larves venant d'éclore, par groupes de 4, dans des boites en aluminium renfermant 120 cm^3 de terreau de feuilles décomposées. Nous avons opéré à 11°, 15°, 20°, et 25° C avec 120 larves dans chaque cas.

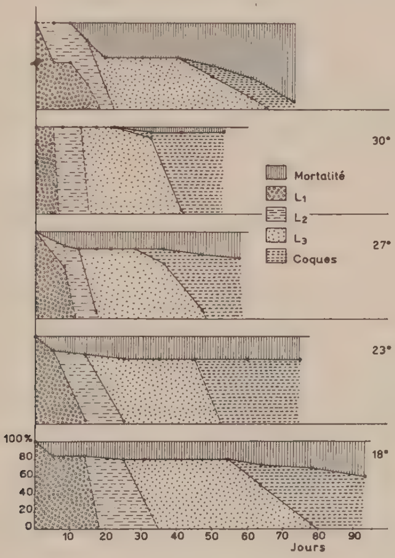

Fig. 1. Action de la température sur le développement des larves de *Epicometis squalida.*

Tous les 15 jours, les insectes étaient pesés de sorte qu'en plus des relevés de mortalité, nous avons pu suivre leur évolution pondérale. A 11° C, quelques individus parvinrent à survivre 6 semaines il n'y eut aucune mue. Le développement noté aux températures plus élevées est résumé dans les tableaux IV, V et VI. D'après ces chiffres, plus la température est forte, plus la croissance est rapide et plus le nombre de coques est grand. Mais dans tous les cas on enregistre une très importante mortalité due en partie aux blessures que s'occasionnent les larves entre elles (ce qui amène la prolifération des mycoses), en partie au cannibalisme et, aussi parfois, au manque de nourriture.

Aussi dans une deuxième série d'élevages faits en 1956, nous avons placé

TABLEAU IV

Croissance des larves de Epicometis squalida *à 15° C.*

Age en jours	Nombre d'insectes				Poids en mg.			
	Ier stade	2 ième stade	3 ième stade	Pré-nymphe	Moyen	Max.	Min.	Stade
2	120				4	7	1	L 1
28	46	3			30	50	20	L 1
43	18	6			75	95	55	L 2
57	6	10			130	230	70	L 2
74	2	5	6		235	365	155	L 3
85	1	1	8		415	660	240	L 3
111			8		435	615	290	L 3
132			3	1	390	445	320	L 3

TABLEAU V

Croissance des larves de Epicometis squalida *à 20° C.*

Age en jours	Nombre d'insectes				Poids en mg.			
	Ier stade	2 ième stade	3 ième stade	Pré-nymphe	Moyen	Max.	Min.	Stade
2	120							
23	8	48			50	120	30	L 2
40	1	21			125	180	55	L 2
			9		195	275	120	L 3
55		5	18		280	490	120	L 3
72		1	19		480	745	260	L 3
83			19		610	955	275	L 3
100			10	5	430	555	265	L 3

TABLEAU VI

Croissance des larves de Epicometis squalida *à 25° C.*

Age en jours	Nombre d'insectes						Poids en mg.			
	Ier stade	2e stade	3e stade	Pré-nymphe	Nymphe	Imago	Moyen	Max.	Min.	Stade
2	120									
23	3	60	4				100	170	45	L 2
40		10					130	190	90	L 2
			47				345	600	110	L 3
55			46	3			390	615	170	L 3
72			17				360	465	250	L 3
				17	4		180	340	105	PN
85			2	9	4	1	200	345	145	PN
100				1	3	1				

chaque larve isolément dans ces mêmes récipients remplis de terreau de foin décomposé. Nous avons opéré avec 44 larves à 18°, 84 larves à 23°, 100 larves à 27°, 40 larves à 30° et 80 larves à 34° C.

Les résultats représentés dans la figure 1, montrent que la température optimum, estimée d'après la vitesse de croissance et le taux de mortalité, est voisine de 30° C, mais les élevages sont très satisfaisants aux températures inférieures.

TABLEAU VII

Durée en jours des stades larvaires de E. squalida.

Température	Premier stade	Deuxième stade	Troisième stade
18°	15	15	35
20°	22	26	40
23°	10	10	32
25°	16	16	30
27°	8	8	26
30°	5	6	24

Le développement ne devient plus lent et le nombre de survivants plus faibles qu'à 34° C. *Epicometis squalida* se comporte donc comme une espèce dont les exigences thermiques sont assez étendues mais situées à un niveau élevé.

Pour différentes températures expérimentées, le tableau VII donne les durées moyennes des trois stades larvaires. Comme chez le Hanneton commun, les deux premiers stades ont sensiblement la même longévité dans toutes les conditions.

La vie larvaire est terminée en deux mois environ. Dans la nature il en est de même, de sorte que les larves ne sont actives que pendant la fin de l'été.

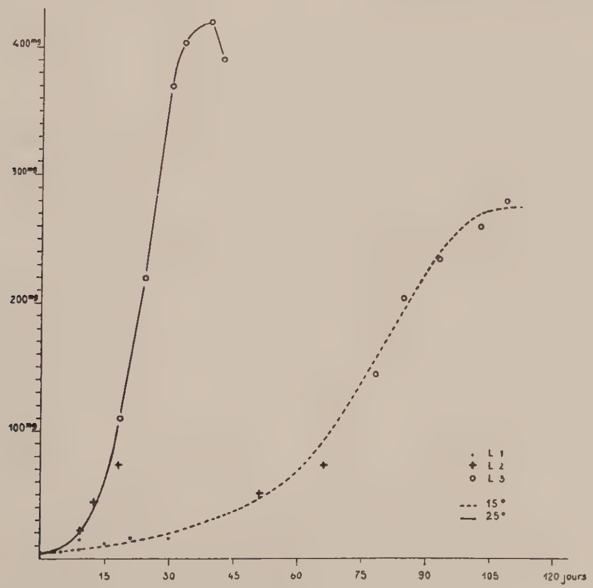

Fig. 2. Evolution pondrale des larves de *Oxythyrea funesta* à 15 et 20° C.

b) *Larves de* Oxythyrea funesta

Les élevages ont été réalisés également dans de petites boites en aluminium remplies de terreau de foin et placées à 10, 15, 25 et 32° C. Chaque boite comprenait une larve âgée au plus de quelques jours.

TABLEAU VIII

Développement des larves de O. funesta *à 15° C.*

Age en jours	Nombre d'insectes			Poids en mg.		
	L1	L2	L3	Max.	Min.	Moy.
1	35			5,5	1	2,5
5	28			11	3	4,5
9	27			15	3	6
15	27			19	5	11
21	25	1		17	8	15
30	17	4		20	6	13
51	3	10		75	32	52
66	2	8	2	91	41	75
78		2	8	245	105	145
85		2	8	255	135	205
93		1	9	280	115	235
103			10	320	160	260
109			10	330	175	280

A 10° C, tous les insectes étaient morts au bout d'un mois sans avoir mué. Les données obtenues à 15 et 20° son rassemblées dans les tableaux VIII et IX et dans la figure 2. A 15°, le développement est plus lent et fait l'objet d'une mortalité plus importante qu'à 25° C. D'autre part, cette température parait insuffisante pour la métamorphose: au bout de 5 mois, il n'y avait encore aucune coque. A 32°, 30 larves du premier stade ne fournirent que 10 coques au bout

TABLEAU IX

Développement des larves de O. funesta *à 25° C.*

Age en jours	Nombre d'insectes						Poids en mg.		
	L1	L2	L3	PN	N	Ip	Max.	Min.	Moy.
1	30						4	1	2
5	30						14	4	9
10	18						18	7	14
		12					30	15	22
13		30					77	16	45
19		18					91	38	75
			12				165	70	95
25		1	29				365	70	200
30			30				535	155	370
33			30				495	235	405
38			28	2			490	380	420
42			21	9			485	300	390
48			1	24	5		235	160	195
55				4	23		210	145	180
60					27		—	—	—
70					8	16	145	110	120
80						22			

de 3 mois. Il semble donc que cette espèce est moins adaptée aux fortes températures que *E. squalida,* son optimum thermique se situant aux environs de 25° C. Le développement larvaire de cette Cétoine est très rapide, pour une larve de *Scarabeide:* 6 à 7 semaines au total.

Influence de l'alimentation

Les larves de Cetonidae vivent généralement dans les matiéres végétales en décomposition: terreau, compost, bois mort, etc.... mais certains milieux paraissent nécessaires pour certaines espéces. Jusqu'à présent, il n'a pas été possible, en France, de déterminer dans quel biotope s'effectuent la ponte et le développement larvaire. Les populations de *O. funesta* sont en général très diffuses et dans le Vaucluse, le climat et la nature du sol rendent très difficiles les sondages dans les champs (GUENNELON, 1958) pour la recherche des larves d'*Epicometis* sp.

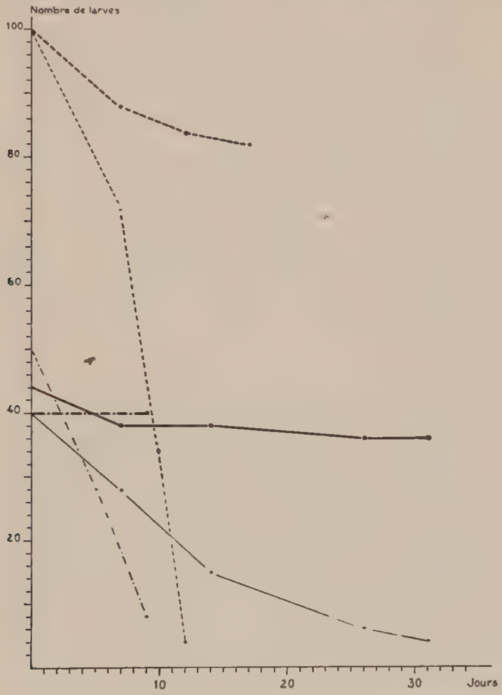

Fig. 3. Influence de la nature du milieu, à différentes températures constantes, sur la mortalité des larves de *Epicometis squalida.*

En trait fin: élevage sur terre — en trait fort: élevage sur terreau de feuilles:

— à 18° C — — — — à 27° C —.—.—.—.— à 30° C

D'après WILKE (1924), *E. hirta* en Allemagne pond en terre et les larves vivent bien dans de la terre à fleurs où elles trouvent suffisamment de radicelles mortes et d'humus pour se nourrir. BOHM (1950) note également que *E. hirta* pond volontiers dans de la terre à condition qu'elle ne soit pas seche.

BEBIC (1954) signale qu'en prairies naturelles, les pontes ne s'effectuent que dans les zones plus meubles, en particulier il en a trouvé beaucoup dans les taupinières.

Nous avons cherché à éprouver la valeur de différents matériaux comme milieu et comme alimentation pour les larves de Cétoines grises en élevage.

a) *Larves de* E. squalida

A différentes températures (fig. 3) nous avons mis des larves de *E. squalida* quelques jours après la sortie de l'oeuf dans des boites contenant de la terre paraissant assez riche en humus et dans des boites pleines de terreau de feuilles décomposées. Comme l'indique la figure 3, la mortalité fut beaucoup plus importante et plus rapide dans le cas de la terre qu'avec le terreau, aux trois températures expérimentées. Dans une deuxième série d'expériences effectuées avec la même espèce, nous avons placé des larves venant de naître dans des substrats variés correspondant à divers habitats possibles dans la nature: terreau d'origine végétale ou animale, résidus provenant d'une décomposition plus ou moins avancée de troncs d'arbres, terre siliceuse ou sciure humide supplémentée par des tranches de carottes. Les résultats de cet élevage conduit à 24° C. sont résumés dans la figure 4 qui met en évidence l'incompatibilité de certains milieux pour le développement de cet insecte et qui fait ressortir nettement l'intérêt que présentent les deux formes de terreau ainsi que le bois en cours de décomposition. Par contre, le bois complétement pourri ne permet pas d'assurer la croissance des larves. Outre des différences dans le nombre des survivants, on enregistre des variations

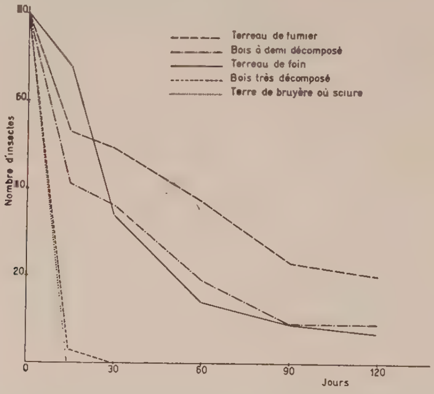

Fig. 4. Action de différents substrats sur le développement des larves de *E. squalida* (à 24° C).

TABLEAU X

Composition des milieux consommés par les larves de Cétoines grises.

	Terreau de foin	Terreau de fumier	Bois décomposé	Terre
Matiére organique	67 %	46 %	32 %	
$P^2\ 0^5$	3,5	5,7	0,7	
N^2	29,6	9,5	6,9	0,13
Humidité relative	53 %	30 %	80 %	

dans la vitesse du développement selon les matériaux consommés: avec les deux terreaux, la deuxième mue commence au bout d'un mois, alors qu'il en faut deux dans le cas du bois décomposé, de même les insectes parfaits sont tous sortis de la coque au bout de 4 mois dans le terreau de fumier, tandis qu'il a fallu attendre 5 mois pour le lot nourri sur le bois en décomposition. D'après l'analyse de ces matériaux faite par la Station Centrale d'Agronomie à Versailles (tableau X), la terreau de foin est le plus riche en matière organique et en azote, mais il est également le plus humide. En tant qu'aliment il semble être à priori, le meilleur, mais en tant que substrat, son humidité parait trop élevée pour assurer une croissance satisfaisante de sorte que le terreau de fumier s'est révélé mieux convenir pour cette espèce, dans les conditions de notre élevage.

b) *Larves d'*O. funesta

Le même type d'expérience fut réalisé avec des larves d'*Oxythyrea funesta* venant de naître, disposées isolément à 24° C dans de petites boites en aluminium renfermant les mêmes milieux que précédemment. Les deux types de terreaux, ainsi que le bois en décomposition, permettent un bon développement, à en juger d'après le nombre d'images formés (tableau XI), qui est du même ordre de grandeur pour ces trois substrats, avec toutefois une supériorité pour le terreau de foin, à la fois par le nombre d'insectes survivants que par la rapidité de leur évolution.

TABLEAU XI

Influence des substrats sur le développement des larves de O. funesta *à 24° C.*

Milieu	Nbre de larves en élevage	Nbre d'imagos formés	% imagos	Délais
Terreau de foin	35	28	80 %	55 à 70 j.
Terreau de fumier	70	45	64 %	60 à 110 j. Moy. 90 j.
Bois à moitié décomposé	30	18	60 %	90 à 110 j.
Terre humifére	35	0	0	—

Le terreau de foin est donc pour ces Cétoines grises le meilleur substrat. Il est probable, en dehors d'une influence éventuelle de la nature de la matière organique et de l'azote dans les différents milieux pris en considération, que *O. funesta* supporte mieux l'humidité que *E. squalida.* Dans ces conditions, la teneur plus élevée en matière organique du terreau de foin serait à l'origine de sa supériorité comme milieu d'élevage.

Dans la terre, les insectes ne subsistent qu'au début de leur existence, pendant les deux premiers stades. Après la deuxième mue, on enregistre une très forte mortalité vraisemblablement due à la famine: les animaux deviennent trop exigeants pour la richesse du milieu en éléments nutritifs, tout au moins en élevage, malgré de fréquents renouvellements du contenu des boites.

DISCUSSION

Parmi les trois espèces de Cétoines grises rencontrées en France, nous avons étudié surtout *Epicometis squalida* Scop. et *Oxythyrea funesta* Poda. *Ep. hirta* Poda a fait l'objet d'essais plus limités à cause de sa moindre abondance, mais c'est sur cette espèce que les renseignements bibliographiques sont les plus nombreux.

La biologie de ces trois insectes est sensiblement la même tant pour l'imago que pour la larve, le cycle évolutif est toujours de 1 an, l'hibernation s'effectue au stade imago immature, les périodes d'activité, la nature de l'alimentation de l'insecte parfait et des larves sont les mêmes, etc.

Cependant, nos essais font apparaître un certain nombre de différences écologiques selon l'espèce considérée. Il en est ainsi par exemple, en ce qui concerne l'action de la température: l'optimum thermique des larves de *E. squalida* est aux environs de 30° C, celui de *O. funesta* de 25° C. Les larves de *O. funesta* ont un développement plus rapide que celles de *E. squalida*: à 25° C celles-ci se mettent en coque au bout de 9 à 10 semaines, tandis qu'il suffit de 6 à 7 semaines pour *O. funesta.* Ces besoins thermiques différents expliquent très vraisemblablement la présence de *O. funesta* dans les régions plus septentrionales que *Epicometis,* tel que le Bassin parisien.

On retrouve ces différences dans les besoins alimentaires. D'une façon générale, les larves des Cétoines grises ne se développent normalement que dans les milieux riches en matiéres organiques et en humus, comme les terreaux. Expérimentalement, elles peuvent vivre dans des arbres en décomposition et la terre se montre satisfaisante lorsque'elle est suffisamment riche en matiéres organiques. Il n'est donc pas exclu que dans la nature elles se trouvent également en pleine terre, à condition que les champs soient suffisamment pourvus en humus et qu'un petit nombre d'insectes ait la possibilité d'exploiter une grande surface.

Mais les larves de *O. funesta* paraissent moins exigeantes que les autres pour le choix de leur nourriture, elles se contentent mieux, par exemple, du bois à demi décomposé.

Enfin, la sensibilité aux maladies est variable selon l'espèce. Le germe le plus virulent est le champignon *Metarrhizium anisopliae* Metchn. qui provoque la „muscardine verte" chez les larves du dernier stade et dans les coques. Il s'attaque surtout aux *Epicometis.* Nous n'avons jamais constaté dans nos élevages d'*Oxythyrea* d'épizooties dues à ce germe. Les cas de mycoses furent toujours isolés. Par contre, certains élevages d'*E. squalida* furent sévérement touchés par cette maladie qui constitue le principal agent de mortalité, en élevage comme dans la nature, pour d'autres espèces de Scarabeides saprophages: *Cetonia aurata* L. et *Oryctes nasicornis* L.

La fréquence élevée des attaques de *Metarrhizium* sur les différentes espèces de ce groupe de coleoptéres est très vraisemblablement due à la nature du milieu où vivent toutes les larves: c'est un substrat riche en humus, humide et maintenu le plus souvent à une température assez forte — ce qui représente un milieu de culture très favorable pour des champignons. Toutefois, il faut remarquer que seul *M. anisopliae* est vraiment important et, d'autre part que les diverses espèces de Cétoines ne réagissent pas de la même facon, ni avec la même intensité. Il y a donc des différences spécifiques nettes dans la sensibilité des trois espèces de Cétoines grises aux mycoses à *Metarrhizium.*

Une autre particularité digne d'être notée dans l'ecologie des Cétoines grises est l'absence de parasites. Jusqu'à présent, nous n'avons pas trouvé d'insectes parasites Hymenoptères ou Diptères, et à notre connaissance il n'y a pas eu d'espèce entomophage signalée dans la littérature pour ces Scarabeidae.

ZUSAMMENFASSUNG

Die Laborzuchten wurden hauptsächlich unternommen, um die Wirkung der wichtigsten ökologischen Faktoren auf die Entwicklung der Larven der grauen Rosenkäfer ("Cétoine grise") zu klären. Die Untersuchungen erstreckten sich in der Hauptsache auf die Arten *Epicometis squalida* Scop. und *Oxythyrea funesta* Poda.

Besonders wurde dabei der Einfluss von Temperatur und andere Umweltbedingungen untersucht. Im Verlaufe dieser Zuchten zeigten sich Unterschiede in der Widerstandsfähigkeit der verschiedenen Arten gegen Temperatureinflüsse, in der Empfindlichkeit für Krankheiten, sowie ihrer Nahrungsbedürfnisse.

O. funesta ist die gegen Umwelteinflüsse am wenigsten empfindliche Art, aber auch die widerstandsfähigste gegen Mykosen. Diese Tatsachen erklären sehr wahrscheinlich die Unterschiede in der geographischen Verbreitung dieser Coleopteren: *O. funesta* findet sich weitaus verbreiteter als *Epicometis.*

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SECONDARY PHYSIOLOGICAL EFFECTS OF DDT IN *GALLERIA* LARVAE

BY

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Secondary physiological effects of DDT leading to death in *Galleria* larvae do not follow a constant pattern. At least three courses have been observed: (1) In the usual toxication, muscular activity is continuous until the insect dies of exhaustion. This is confirmed by the evidence that DDT does not hasten death if muscle action is blocked by braconid venom. Adenosine triphosphate (ATP) is not depleted until after death. (2) DDT-induced hyperactivity predisposes the insect to non-specific "natural" mortality. (3) DDT induces regurgitation. Under special environmental conditions that permit larvae to become covered with regurgitated fluids, they quickly become prostrate. Prostrate larvae show a loss of ATP. With different treatments, prostrate larvae may (a) die with no further activity, (b) become active and follow the usual course of DDT toxication, or (c) dramatically recover from prostration and the effects of DDT. The nature of these events is discussed, but not fully explained.

It is generally agreed that DDT acts primarily on nervous cells and tissues. DDT is known to unstabilize peripheral sensory elements, but its action on the more central nervous elements has not been clearly defined (see review by Kearns, 1956). The secondary events leading to death, which have not been adequately explored, are the subjects of inquiry here reported.

Hyperactivity of muscle is the usual consequence of the neural effects. Muscle studies have served chiefly to exclude this tissue as the primary site of DDT action. Merrill, Savit & Tobias (1946) observed in DDT poisoned *Periplaneta* that cyclopropane suppressed muscle activity during anesthesia, but did not permanently eliminate symptoms of poisoning. Such anesthesia also stopped the depletion of carbohydrates observed in the usual course of DDT poisoning. Thus carbohydrate loss was attributed to muscle hyperactivity rather than to direct action of DDT. Treated insects went on to die even though their muscle activity had been spared during anesthesia. This has been interpreted as assigning to muscle a less important role in DDT poisoning than was probably intended by the original writers.

Winteringham (1956), using houseflies, has confirmed that temporary cyclopropane anesthesia does not conclusively terminate symptoms of poisoning. He also found a loss of adenosine triphosphate (ATP) in flies made prostrate by DDT action. He further observed that some relief from DDT poisoning could result from injection of glucose into affected flies. Whether loss of ATP was a direct effect of DDT or a consequence of muscle exhaustion was not resolved.

Cyclopropane produces a general anesthesia. More specific inactivation of muscle for extended periods without stopping other bodily functions should better differentiate between the direct effects of DDT and the metabolic changes arising from the hyperactivity of muscle. This can be done in larvae of *Galleria mellonella* (L.) by injection of extracts containing the active principle used by braconid parasites in paralyzing their prey (BEARD, 1952). This provides a system favorable for studying the role of muscle activity in DDT poisoning. This paper reports the results of such a study.

MATERIALS AND METHODS

Late-stage larvae of *Galleria* were used as the test organism primarily because their muscles are readily paralyzed by venom of *Microbracon hebetor* Say (= *Habrobracon juglandis* Ashmead). *Galleria* larvae are reared in the laboratory on a food medium composed of dog food, a proprietary cereal for infants, and honey. *Microbracon* is readily reared as a predator-parasite on *Galleria.* The active principle in the wasp that causes paralysis in the larva is water soluble and relatively stable. This permits easy extraction by grinding whole wasps in water and filtering. The filtrate is used directly. Although the potency can be judged by bioassay, this was not required in these experiments. Administration of this and other chemicals was by injection into the body cavity. The volume injected (usually 3 microliters per larva) was controlled by a micrometer screw injection device and hypodermic syringe.

ATP was determined by an adaptation of the technique described by KORNBERG (1950). Weighed samples of *Galleria* larvae were homogenized in cold perchloric acid. The suspension was filtered, neutralized, and centrifuged (all under refrigeration). Aliquots were then analyzed on a Beckman spectrophotometer (at 340 mμ) for reduced triphosphopyridine nucleotide (TPN) on the principle of the following reactions:

$$\text{glucose} + \text{ATP} \xrightarrow[\text{Mg}^{++}]{\text{hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

$$\text{glucose-6-phosphate} + \text{TPN} \xrightarrow{\substack{\text{glucose-6-phosphate}\\ \text{dehydrogenase}}} \text{6-phosphogluconate} + \text{TPNH} + \text{H}^{+}$$

Correction was made for small amounts of glucose-6-phosphate present in the system.

The results of some experiments were found to be so dependent upon methods of treating and handling the larvae that other details of technique must be discussed in direct relation to the observations.

RESULTS AND DISCUSSION

Toxicity of DDT to Galleria *larvae*

DDT is not highly toxic to *Galleria* larvae. The LD_{50} for DDT in oil solution was found to be 74 μgm/gm body weight for body cavity injections, 151 for gut injection, and 834^{+} for topical application (BEARD, 1949). The last figure was obtained by extrapolation and represents a figure impossible to achieve in practice. In fact, *Galleria* larvae may be said to be insensitive to DDT applied externally.

They may be dipped in 0.25 per cent DDT emulsions or thoroughly dusted with 10 per cent DDT dust without showing characteristic symptoms of DDT poisoning. Hence formulation is not a factor in this topical insensitivity.

Symptoms and usual course of DDT toxication

Larvae injected with lethal doses of DDT normally follow a predictable sequence of activity. As first symptoms of poisoning, locomotion becomes jerkey. This quickly worsens to convulsive movement without purposeful orientation. This action consists of gross flexing movements. Hyperactivity continues without interruption, lasting days or even weeks. As time goes on, movement becomes weaker. The insects obviously lose weight, and those surviving the longest become small and shrivelled. As long as any body muscle remains contractile, twitching is evident. Death is assumed when all activity ceases. Prostration, such as is observed in some other insects, does not usually occur.

The time sequence of toxic symptoms is more rapid if DDT is administered as an emulsion rather than in oil solution. This can be explained on the basis of more rapid partitioning to receptive tissues in the insect.

When DDT is injected into larvae paralyzed by braconid toxin, no visible symptoms of poisoning appear. Nerves are characteristically stimulated, but muscle action is blocked (BEARD, 1952). Hence DDT-treated paralyzed larvae are indistinguishable from paralyzed larvae receiving no DDT (Figure 1). The death rate of paralyzed DDT-treated larvae more closely follows that of paralyzed larvae than that of active larvae treated with the same dosage of DDT. This is illustrated in Figure 2, in which the cumulative mortality rates for three groups are shown. In the two groups of paralyzed larvae muscle action was blocked for one week in the larvae surviving that time. These larvae continued development if DDT was absent; pupation seemed normal in spite of any inanition suffered during paralysis. Similar larvae treated with DDT showed no symptoms of toxication during paralysis; when muscle activity resumed, however, typical symptoms appeared and the normal course of DDT toxication continued to death. In contrast active larvae treated with DDT all died within a few days.

From these observations two conclusions can be drawn. One, that DDT does not hasten death in this insect if muscle action is blocked, and two, that after paralysis (of even a week's duration) DDT was still present in sufficient amounts to cause symptoms and consequent death. Similar interpretation can be placed on the cyclopropane anesthesia experiments of MERRILL *et al.* (1946).

WINTERINGHAM (1956, 1957), having observed a loss of ATP in prostrate flies, raised the question as to whether this was a direct action of DDT or a consequence of muscle activity. This can be answered for *Galleria* larvae. If ATP levels remained normal in DDT-treated larvae in which muscle activity was blocked, it could be concluded that DDT had no direct action on ATP. If prolonged convulsions in DDT-treated larvae without muscle block failed to deplete ATP, it could be concluded that DDT had neither a direct or indirect action on ATP. Both of these conclusions can be drawn from data presented in Table I, which gives results of ATP determinations in groups of larvae representing the appropriate physiological states. These results are composite data drawn from three simi-

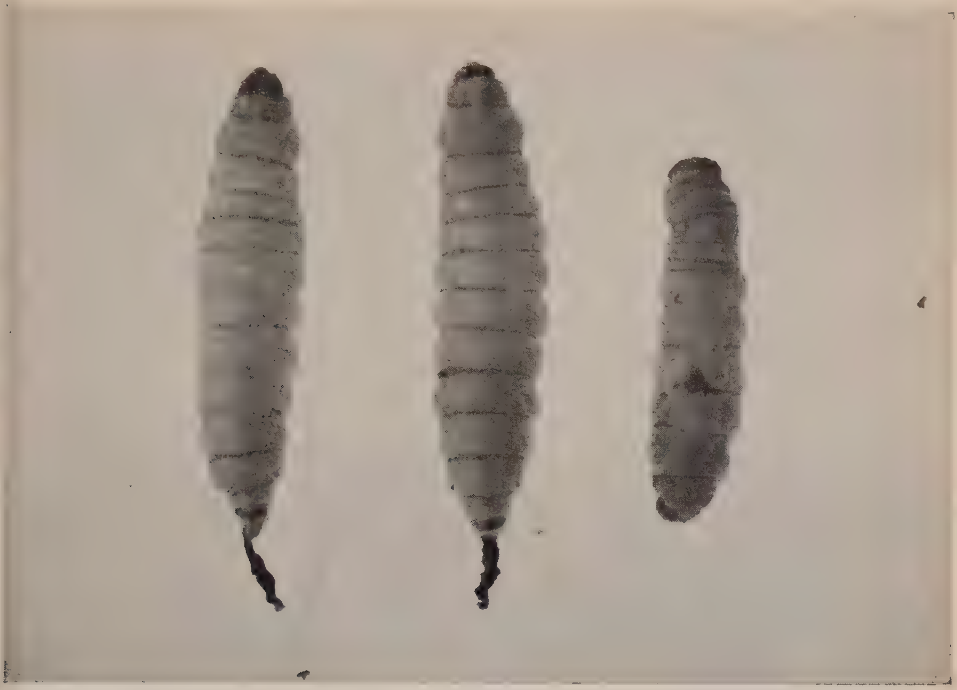

Figure 1. Appearance of *Galleria* larvae four days after treatment. Left and center: larvae received equal dosages of paralyzant. Center and right: larvae received equal dosages of DDT. Note defecation by two paralyzed larvae.

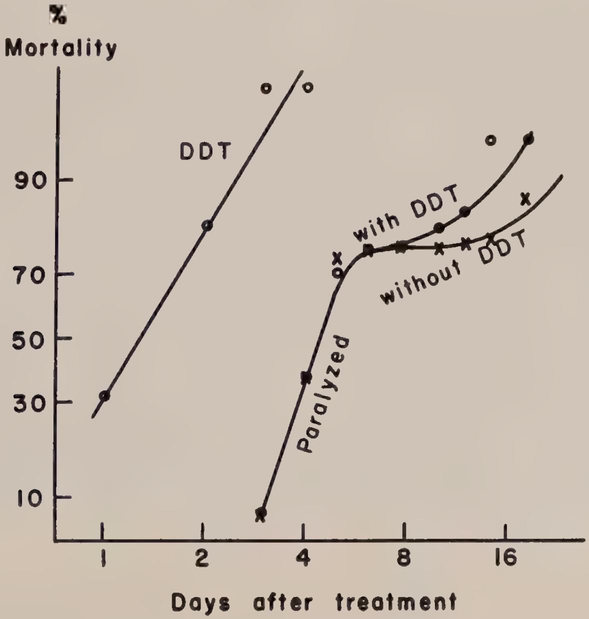

Figure 2. Cumulative mortality trends of *Galleria* larvae, treated with (1) DDT alone, (2) paralyzant alone, and (3) DDT and paralyzant. Activity in paralyzed larvae resumed after about 8 days.

R. L. Beard: *Effects of DDT in* Galleria *larvae*

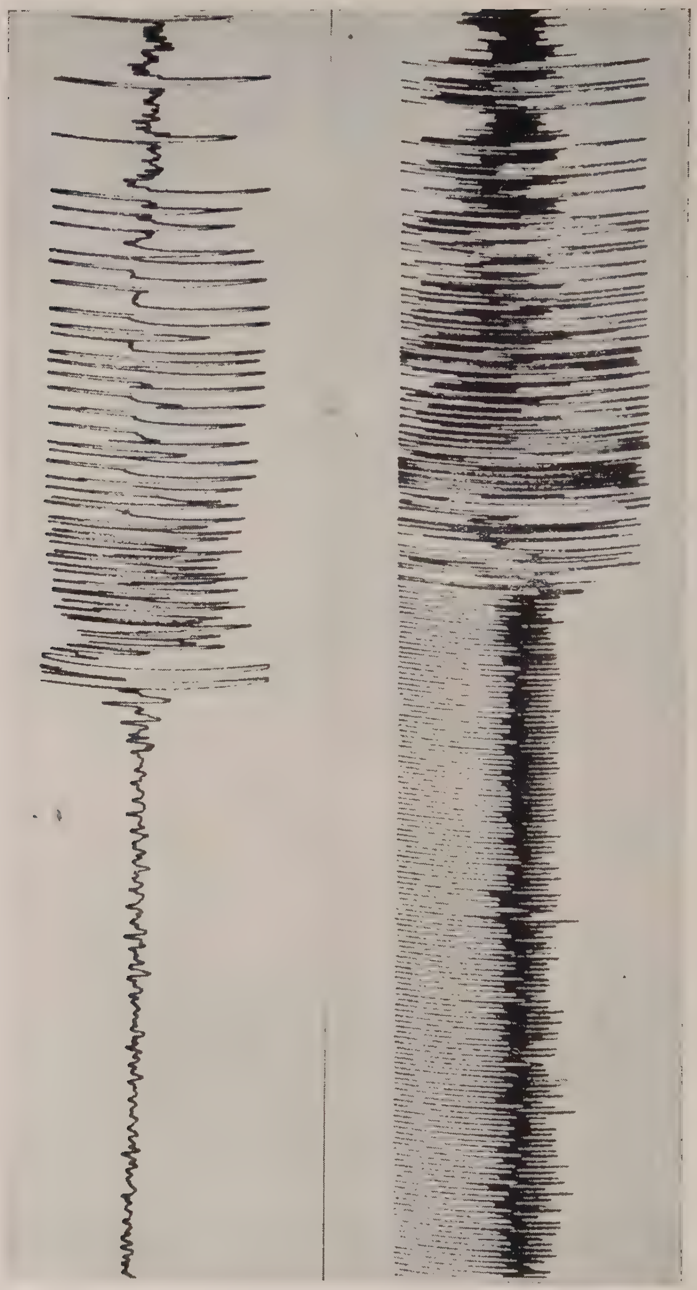

Figure 3. Recordings of foregut activity. Upper trace shows quiescent gut activity followedby DDT induced spasms. Lower trace shows rhythmic (peristaltic) gut activity followed by DDT induced spasms. Speed of upper recording 2.5 times that of lower recording.

R. L. Beard: *Effects of DDT in* Galleria *larvae*

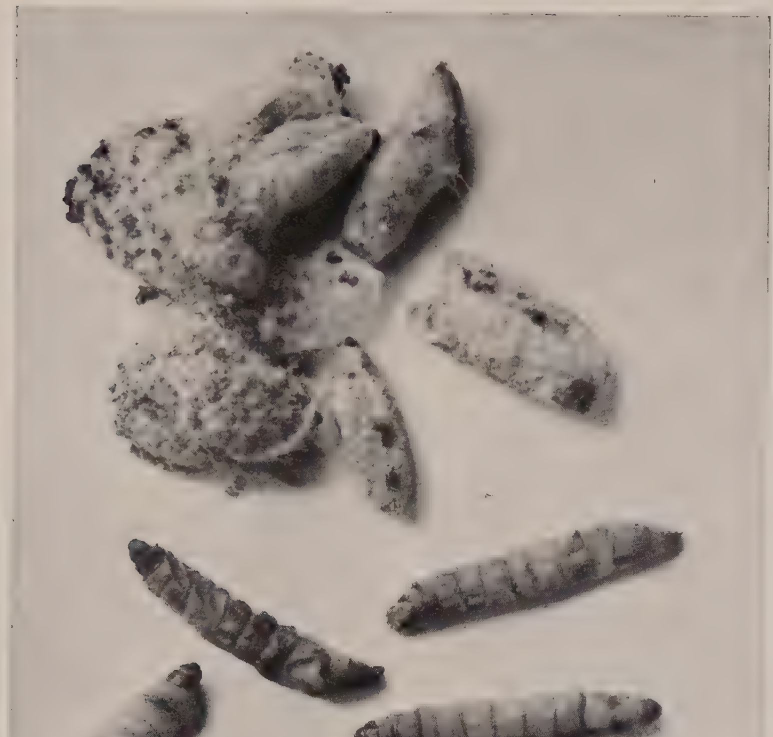

Figure 4. DDT-treated larvae after prostration (24 hours after treatment). Group on left all died. Group on right recovered and spun cocoons after receiving ATP (0.6 µmole per larva).

lar but separate experiments. This tabulation is justified as all positive results are sufficiently similar to make the conclusion clear that in *Galleria* larvae under these conditions DDT does not deplete ATP, either directly or because of muscle activity. It is only when the larvae die and post-mortem changes occur that ATP is depleted. Hence this loss of ATP is a consequence of death and not a cause.

TABLE I

ATP FOUND IN HOMOGENIZED WHOLE LARVAE

Effect of DDT, with and without muscle-block

Condition	µmole ATP per gram larvae
Untreated	1.33
Untreated	1.41
Untreated	1.51
DDT-treated, convulsing 1 day	1.35*
DDT-treated, convulsing 3 days	1.49*
DTT-treated, muscle-block, 1 day	1.55
DDT-treated, muscle-block, 3 days	1.32
Killed by DDT	0.00

* Corrected for differential weight loss (dry tissue-fat-water) associated with marked loss in total body weight.

This means that this course of toxication is different from that shown by the housefly as described by WINTERINGHAM. The chief difference is that instead of passing through the prostate stage, the insect convulses to exhaustion and death.

Intensified "natural" mortality

The course of toxication just described is not invariable. All DDT-treated larvae do not survive to reach the emaciated state resulting from continuous muscle activity. Some die at earlier stages, their tissues break down, and they turn black. This condition, also appearing as the occasional death in untreated test groups, is probably induced by diverse causes not directly associated with the primary action of DDT nor with direct consequences of muscle activity. It is reasonable to conclude that DDT toxication weakens the insect and predisposes it to "natural" mortality factors.

Modified course of DDT toxication

At times a curious departure from the usual sequence of symptoms was observed in DDT-treated larvae. Soon after convulsions began, the larvae acquired an oily appearance and within a few hours became rigidly prostrate. If left undisturbed, none survived. If treated in certain ways, activity was restored and recovery was possible. Hence this prostration is not equivalent to death. Prostrate larvae show no heart beat nor are their muscles responsive to electrical stimulation.

It was established that this condition resulted when larvae, after DDT treatment, were placed in a closed container with no other substrate. Treated larvae placed uncrowded in an open vessel or on an absorbent substrate did not acquire the oily appearance nor did they become prostrate. They followed the normal course of toxication. Ligating treated larvae behind the head provided convincing evidence that the oily appearance was caused by the regurgitated gut contents becoming smeared over the larvae by their convulsive movements. Mutual contact of the larvae served to distribute the fluid and also to aggravate movement.

As this prostration suggested a type of DDT toxication more like that in flies, ATP determinations were made of three series of larvae treated with DDT under conditions leading to prompt prostration. At intervals of 10, 40, and 160 minutes after injection of lethal dosages of DDT larvae were prepared for ATP analysis. These samples are compared with untreated controls in Table II.

TABLE II

ATP FOUND IN HOMOGENIZED WHOLE LARVAE

Effect of Prostrating Conditions

Conditions	μmole ATP per gram larvae
Normal	1.41
DDT convulsions for 10 minutes	1.40
DDT convulsions for 40 minutes	1.29
DDT convulsions for 160 minutes	0.56

A progressive loss of ATP is evident in these pooled samples. It was noted that of 10 larvae convulsing for 40 minutes one had become prostrate, and of the 10 convulsing for 160 minutes six had become prostrate. The ATP content is thus reduced in proportion to the extent of prostration. That all ATP in a prostrate larva is not lost is demonstrated in a confirming test on 10 prostrate larvae in which the ATP content was 37 per cent of normal.

In this type of toxication, prostration and death occur too promptly to be attributable to gross exhaustion. The loss of ATP can certainly be attributed to secondary effects of DDT poisoning.

From the foregoing discussion it is apparent that the primary action of DDT triggers a series of events the course of which depends on other things. What might be called the normal course of toxication is that of hyperactivity leading to a slow death from gross muscular exhaustion. In this event, DDT has no effect on ATP, either directly or indirectly.

This course may be interrupted by non-specific contributing factors which unpredictably cause an earlier death. There is nothing to identify this type of death with a characteristic action, so it is considered an intensified natural mortality.

The course of toxication precipitated by regurgitation and concluding with prostration is characteristic of particular circumstances. Here ATP is definitely reduced, but in view of other analyses, this cannot be a direct action of DDT.

The prostrate condition

An understanding of the prostrate condition mentioned in the preceding section is not essential to the conclusion that DDT toxication may follow different path-ways in *Galleria* larvae. This prostration, however, is a peculiar phenomenon and although its nature and causes cannot be fully explained, it deserves more than passing comment.

Regurgitation

Regurgitation, a necessary precursor for prostration, is a direct consequence of the primary action of DDT; it is the result of spasms of the foregut. Electrodes, inserted in the foregut (via the mouth) and the body cavity and led to suitable amplifiers and a recording galvanometer, permit recording of electrical activity associated with gut movement. Such recordings are illustrated in Figure 3. The gut can be quiescent, as indicated by an irregular trace of low amplitude, or it can contract rhythmically, as indicated by a regular trace of greater amplitude. In either case, injected DDT causes spasms. Any rhythmicity is modified, and regurgitation of the foregut contents accompanies the onset of spasms. Regurgitation may be reinforced by the squeezing action of the body wall muscles. Action of DDT is more rapid if injection is in the body cavity than directly in the gut. This and the fact that regurgitation coincides with the beginning of convulsive movements, indicates that these actions have a common origin in the neural effects of DDT. Just what nervous elements are affected to induce spasms are not known.

Regurgitation alone does not result in prostration. This is indicated by the fact that prostration can be prevented by absorbing the regurgitated fluid on filter paper or by washing the smeared larvae prior to prostration. After larvae become prostrate, washing has no effect.

The oily appearance of the affected larvae and the subsidence of activity suggests a kind of "drowning". The prostrate condition is not simulated physiologically by immersion of larvae in water. DDT-treated convulsing larvae when in contact with water also become rigidly prostrate very quickly, and continued immersion undoubtedly results in true drowning. After short periods of immersion (10 minutes), however, larvae resemble the DDT prostrate larvae only superficially. Water-immersed larvae show a high rate of survival, their muscles are irritable to electric stimulation, and their ATP content is normal. These characteristics do not apply to the DDT-prostrate larvae.

A few spiracles are plugged by particulate matter in larvae under prostrating conditions, but similar plugging by wax in healthy larvae is insufficient to induce prostration. Larvae under prostrating conditions subjected to high oxygen tensions continue to become prostrate. Reduced oxygen tensions do not hasten the onset of prostration.

The regurgitated fluid by itself is not sufficiently toxic to account for prostration. Healthy larvae or paralyzed larvae placed among larger numbers of convulsing larvae in close confinement become smeared with the fluid, but remain unaffected after the DDT-treated larvae become prostrate. It appears, then, that a combination of the internal physiology, altered by DDT and the effect of the regurgitated fluid is required for prostration. Although recovery from prostration is possible, the interval before death, during which a prostrate larva can recover, is probably short. This interval has not been determined because the complex conditions permitting recovery are not fully known.

The loss of ATP suggests that if this is a direct cause of prostration, ATP might alter the course of toxication if administered just before or at the time of prostration. An exploratory test resulted in dramatic recovery of larvae under conditions otherwise leading to prostration and early death. DDT-treated larvae injected with a high dose of ATP completely recovered and spun cocoons within 24 hours. During this time other DDT-treated controls all died (see Figure 4). Repetitions of this test, with known dosages of ATP led to different degrees of success. Table III illustrates the results of one test in which the effect of ATP, injected prior to prostration, varies with the dosage of DDT applied. Obviously, the larvae without ATP progressively deteriorated; those with ATP improved (except the group receiving the most DDT).

TABLE III

DDT Symptoms in larvae with and without ATP

μgm. DDT/larva	Relative effect of DDT*	
	No ATP	0.6 μmole ATP / larva
	3 hour evaluation	
15	52	40
7.5	47	24
3.75	28	15
	24 hour evaluation	
15	54	46
7.5	50	16
3.75	55	4

* Total effect on 15 larvae in each group. Individual effect based on following criteria: 0 — no effect; 1 — jerky ambulation; 2 — convulsions; 3 — prostrate with some muscle action; 4 — complete prostration or death. Maximum score = 4n = 60.

Other chemicals were evaluated in the same way as ATP, but none produced such dramatic recoveries from DDT poisoning. Glucose had some ameliorative action. In one test DDT-treated larvae injected with 0.6 μmole of glucose showed reduced symptoms, scoring 19 as compared with 57 in control larvae, by the criteria outlined in Table III. Higher concentrations of glucose actually hastened death. Adenosine, inosine, and inosine triphosphate also partially alleviated the prostrate condition. No reduction in DDT symptoms followed injections of adenosine diphosphate. Because of inconsistent results, quantitative comparisons of these several chemicals are not helpful, but it seems evident that ATP is not specific. Thus the reduction of ATP *per se* is not the sole cause of prostration.

As ATP does not consistently alleviate the DDT toxication, it is clear that a complex set of reactants exist. If conditions are in proper balance, ATP can effect recovery. More often, this balance is not appropriate, and ATP has little or no effect. Until the proper conditions are known, recovery seems largely fortuitous. Table III suggests that the amount of DDT available to act is an important condition. Direct antagonism of ATP and DDT is unlikely, as larvae injected simultaneously with both chemicals become prostrate if placed in proper environment. No combination of chemical treatment, washing, and changed environment has been tried that insures consistent recovery. So this phenomenon of prostration remains a puzzle.

ACKNOWLEDGEMENT

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ZUSAMMENFASSUNG

Die zum Tode von *Galleria*-Larven führenden sekundären physiologischen Wirkungen von DDT zeigen kein einheitliches Bild. Wenigstens drei Wirkungsarten wurden beobachtet.

(1). Bei gewöhnlicher Vergiftung fahren die Muskeln fort sich zu bewegen, bis das Insekt vor Erschöpfung stirbt. Dies wird dadurch bestätigt, dass DDT den Tod nicht schneller bewirkt, wenn die Muskeln durch den Giftstoff von Braconiden an der Bewegung gehindert werden. Der ATP (Adenosin Triphosphat) -Gehalt bleibt nach dem Tode normal.

(2). Die von DDT verursachte Hyperaktivität bewirkt, daß das Insekt irgendwelchen „natürlichen" Todesursachen leichter erliegt.

(3). DDT verursacht Erbrechen. Unter besonderen Umständen werden die Insekten, die sich mit erbrochenen Flüssigkeiten besudeln, bald gelähmt. Gelähmte Larven zeigen einen Verlust an ATP. Je nach der Behandlung können gelähmte Larven a) sterben, ohne sich weiter zu bewegen, b) wieder aktiv werden und den gewöhnlichen Verlauf von DDT-Vergiftung zeigen oder c) sich erstaunlicherweise von der Lähmung und den Wirkungen des DDT erholen.

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PUPARIUM FORMATION IN *CALLIPHORA VOMITORIA:* A HITHERTO UNSUSPECTED FUNCTION OF THE EPICUTICLE

BY

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Removal of the epicuticle from larvae of *Calliphora vomitoria* causes the underlying procuticle to become deeply coloured owing to the oxidation of its dihydroxyphenols. This oxidation is non-enzymatic. In the intact larva the epicuticle protects the phenols from spontaneous oxidation so that they are available later for use in puparium formation. The changes which take place in the procuticle as puparium formation is approached are described, and their significance in relation to the onset of phenolic tanning is discussed.

It has recently been shown (DENNELL, 1958a) that dihydroxyphenols are formed in the larval cuticle of *Calliphora vomitoria* L. as a result of the non-specific hydroxylation of aromatic amino acids. The phenols are used to harden the cuticle when it is converted into the puparium (DENNELL, 1958b). The process of phenol production begins as the larval crop is emptied in preparation for pupation, so that for 2—3 days before the puparium is formed phenols are present in the cuticle. A polyphenol oxidase is also present, but in spite of this only slight oxidation takes place before the pupal contraction, and in the epicuticle only. In discussing the reason for this it was suggested (DENNELL, 1958b) that protection of phenols against premature oxidation might be due to the action as an oxidase inhibitor of the resorcinol which is produced during the hydroxylation process.

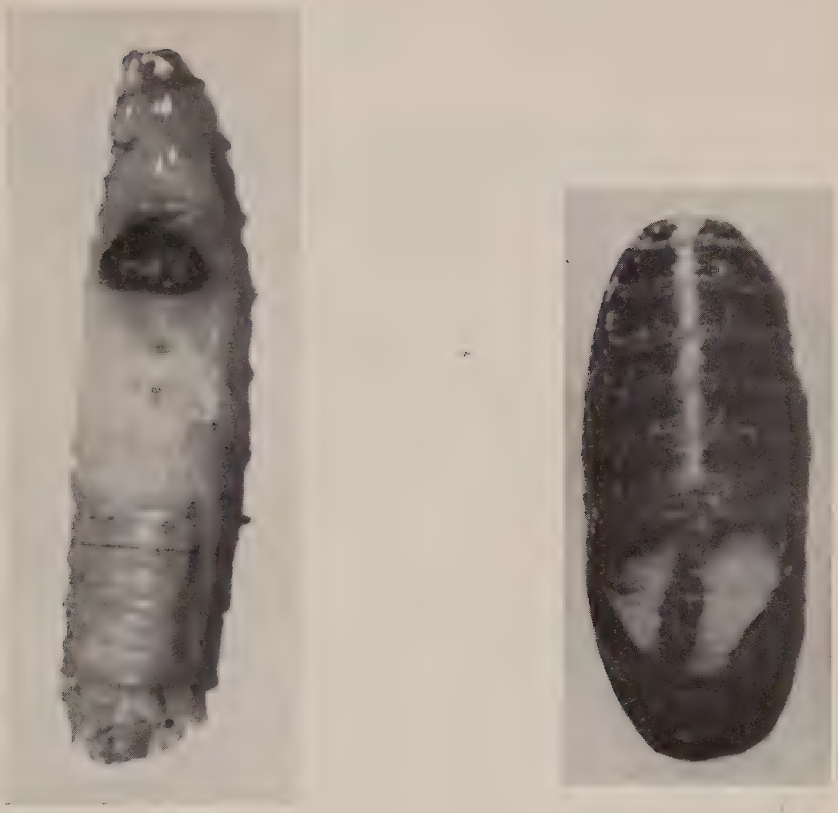
But if the cuticle is abraded the resulting scratches become brown, apparently due to the oxidation of dihydroxyphenols. This suggests that in the intact larval cuticle phenols and oxidase may, owing to the location of the oxidase in the epicuticle, be physically held apart, and come into contact only when the epicuticle is damaged. Further investigation showed, however, that this is not the cause of the darkening of the scratches, and that a hitherto unsuspected function must be ascribed to the epicuticle.

The histochemical methods employed in the observations reported here have already been described (DENNELL, 1946, 1947; DENNELL & MALEK, 1955a, b).

THE EFFECT OF REMOVING THE LARVAL EPICUTICLE

Preliminary investigations showed that the browning of the abraded cuticle is not enzymatic. Larvae were rubbed with emery and immediately afterwards killed by heating in air at 100° C for 5 mins. Brown streaks and spots developed just as they do in unheated larvae. Their appearance can only be explained as being due to the spontaneous oxidation of cuticular phenols when they are exposed to the

R. Dennell: *Puparium formation in* Calliphora



a.

b.

Figure 1.
a. The darkening of an area of larval procuticle caused by removal of the epicuticle.
b. The effect on the puparium of cautery and epicuticle removal from the larval cuticle. The white areas have been cauterized, and the longitudinal black band separating them is the intervening cauterized area from which the epicuticle has been removed.

air by abrasion of the epicuticle. If this is true, then the selective removal of the epicuticle so as to expose the procuticle is also to be expected to cause browning.

To test this, sheets of epicuticle were removed from considerable areas of the cuticle of living larvae in which the crop had just been emptied. The surface of the cuticle was pinched up with fine forceps, and the ridge so formed gently dragged. This caused the epicuticle to tear away from the remainder of the cuticle, and it was then quite easy to strip away extensive areas. Like that of *Sarcophaga* (DENNELL, 1946) the epicuticle consists of two layers, but histological examination shows that not only these but also the first few laminae of the procuticle were removed. Henceforward in this paper unqualified mention of the epicuticle is to be taken as referring to the epicuticle together with these laminae.

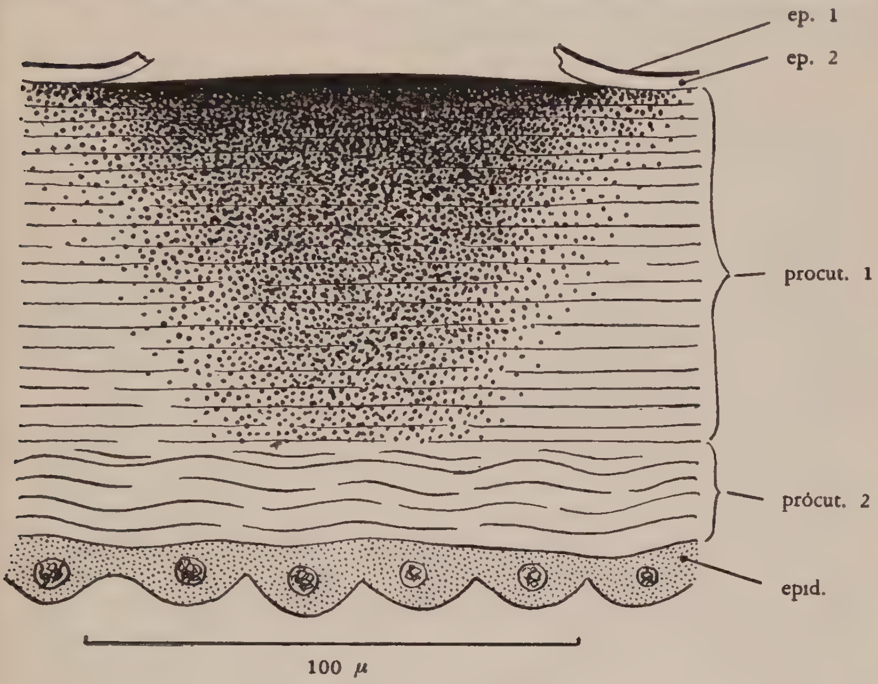


Figure 2.
Section through the dark area seen in Fig. 1a. Darkening extends through the future exocuticle (*procut.* 1) but not into the future endocuticle (*procut.* 2.)
ep. 1., outer layer of epicuticle; *ep. 2.*, inner layer of epicuticle; *epid.*, epidermis.

The result of removing the epicuticle from the larval cuticle was that the exposed procuticle began to darken after 2—3 hrs. at 25° C. After 12 hrs. it had become dark brown or black (Fig. 1, a). Sections showed that the darkening was most intense at the surface (Fig. 2), although it extended inwards throughout the whole thickness of the future exocuticle. The inner zone of procuticle, which does not darken in the puparium, showed no change.

These results confirm the conclusion, reached by heating abraded larvae, that the browning of the exposed procuticle cannot be enzymatic, for the cuticular oxidase is necessarily removed with the epicuticle. The epicuticle must be regarded as a barrier preventing the direct access of atmospheric oxygen to the cuticle, so that oxidation of phenols does not normally take place. This is confirmed by coating the exposed procuticle with rubber solution or celloidin. Darkening is greatly delayed and reduced in intensity. The paradoxical conclusion is therefore reached that although the epicuticle carries the oxidase which promotes the oxidation of phenols its impermeability prevents the supply of the necessary oxygen. It is evident that some change must take place at or about the pupal contraction to allow of the rapid oxidation of phenols during the hardening process.

Cautery and epicuticle removal in late larvae and puparia

Removal of the epicuticle from larvae older than those referred to in the previous section had the following results. When the larvae were within 1—2 hrs. of pupation, the exposed procuticle darkened simultaneously with the remainder of the cuticle. Its colour became that of the puparium, and not the dark brown, almost black, seen in the larva. When light brown puparia were used it was found that up to this stage only the epicuticle had undergone natural darkening. Its removal revealed contrasting white procuticle, which soon darkened, its final colour being that of the general surface of the puparium. As the darkening of the puparium is undoubtedly enzymatic, it is to be expected that darkening of the exposed procuticle, unlike that seen in the larva, is also enzymatic. This was confirmed by the following experiment.

TABLE I

The colour developed by the procuticle after various operations on different developmental stages. 1, 2—3 hours after operation, 2, in the fully formed puparium.

Developmental stage at time of operation	Operation, and colour developed by procuticle					
	Epicuticle removed		Cuticle cauterized		Cuticle cauterized and epicuticle removed	
6 day larva	1. Brown	2. Almost black	1. None	2. None	1. Brown	2. Almost black
7 day larva	Brown	Almost black	None	None	Brown	Almost black
Larva 1—2 hrs. before pupal contraction	Pale redbrown	Chestnut brown	None	None	None	Pale brown
White puparium	Light chestnut brown	Chestnut brown	None	None	None	None
Early (light brown) puparium	Light chestnut brown	Chestnut Brown	None	None	None	None

A strip of epicuticle was removed from an area of the early puparium which had been cauterized with a bot needle to destroy the oxidase. No further darkening of the cauterized area took place, even where the procuticle was exposed. This contrasts sharply with the result of a similar operation on the larval cuticle, in which removal of the epicuticle causes darkening in the cauterized area, so that when the larva pupates the exposed procuticle is seen as a dark streak crossing the white cauterized area (Fig. 1b). It is clear that darkening of the puparial procuticle, whether naturally or after removing the epicuticle, is enzymatic.

The results of a number of experiments on the effects of cautery and removal of the epicuticle on the cuticle of different development stages are summarized in Table I.

HISTOCHEMICAL OBSERVATIONS ON FROZEN SECTIONS OF LARVAL AND PUPARIAL CUTICLES

The procuticle and epicuticle of early larvae of *C. vomitoria*, like those of *Sarcophaga* (DENNELL, 1946), show iso-electric points in the neighbourhood of pH 3,5 and 5.1 respectively. But when this observation, made by the method employed by YONGE (1932), is repeated at intervals during larval and puparial development it is found that not long before the pupal contraction the iso-electric point of the procuticle rises to the level characteristic of the epicuticle. At the same time the procuticle becomes Millon-positive, and, as in *Periplaneta* (DENNELL & MALEK, 1955a), it may be concluded that it has become impregnated with a protein different from that originally present.

Up to this time the use of Sudan Black B does not reveal the presence of lipids in the procuticle or epidermis, although the epicuticle colours readily. Immediately before the pupal contraction, however, the cytoplasm of the epidermal cells is found to be loaded with Sudan-staining globules. In the white pupa the globules are smaller and less abundant, and the future exocuticle takes up Sudan Black lightly but distinctly. It therefore seems that lipid material passes from the epidermis to the future exocuticle as the pupal contraction takes place. The amount of this material accumulating in the puparial cuticle is greater than is indicated by the above observations, for after treatment with Diaphanol for 10 days colouring with Sudan Black is much more intense. Apparently much of the lipid becomes masked as it does in *Periplaneta* (DENNELL & MALEK, 1955a). Also as in *Periplaneta,* it is readily shown by the Liebermann-Burchardt reaction to be a sterol.

These are not the only changes which take place in the preparation of the cuticle for puparium formation. Application of the 'Nadi' reagent, used by DENNELL (1946) to demonstrate the presence of an oxidase in the epicuticle of larvae of *Sarcophaga*, gives evidence of a re-distribution of the cuticular oxidase during the pupal contraction. As in *Sarcophaga*, the oxidase is identified first in the epidermis, and then is found in the epicuticle, having disappeared from the epidermis. It remains confined to the epicuticle until the pupal contraction, and then begins to spread inwards into the procuticle. This process continues until the whole of the future exocuticle contains oxidase. The inner region of procuticle, which does not harden, remains without oxidase. It is interesting that a similar inward migration of oxidase takes place in the developing cuticle of *Periplaneta* (Mr. J. KENNAUGH, private communication).

A further change is that whereas the oxidase of the larval epicuticle is completely inhibited by .002 M KCN, that of the puparial exocuticle in which tanning has begun still oxidises the 'Nadi' reagent in the presence of .05 M KCN. In the puparium of *Sarcophaga* (DENNELL, 1947) inhibition of the oxidase was not complete in the presence of cyanide.

It is evident from these observations that the puparial procuticle differs considerably from that of the larva, and that the epidermis is active in the white pupa. The experiment next to be described shows that the epidermis is important in the tanning process.

THE EFFECT OF DESTRUCTION OF THE EPIDERMIS ON PUPARIUM FORMATION

In an attempt to discover whether impregnation of the exocuticle with sterol is an essential step in the tanning of the puparium, the epidermis was removed from restricted areas of larvae approaching pupation. A needle was inserted through the flank and its point passed forwards in the haemocoele for a distance of a few millimetres. The point was then pressed outwards and scraped against the epidermis. The larvae survived the operation and pupated at the expected time.

No externally visible result followed removal of the epidermis when it was performed less than 16 hrs. before pupation, but when effected earlier the result was unequivocal. The cuticle above the operated area either did not tan, or coloured only slightly. Sections showed that the epidermis had been effectively removed, and that the exocuticle had not been impregnated with sterol. More than this, however, the normal inward migration of oxidase had not taken place, suggesting a connexion between the presence of sterol and the passage of oxidase.

DISCUSSION

As is well-known, the cuticle of insects is unsuited to cutaneous respiration. Only limited gaseous exchange takes place through the cuticle of blowfly larvae, as shown by the fall in oxygen tension inside the larva from 15% tot 2.4% which takes place when both anterior and posterior spiracles are occluded by ligatures (FRAENKEL & HERFORD, 1938). Since it is the epicuticle which is chiefly responsible for impeding the passage of water, it is natural to suppose that this layer is also effective in limiting gaseous exchange. The observations reported here are in agreement with this view, and support the conclusion reached by FRAENKEL (1935) that the oxygen used in the enzymatic oxidations involved in puparium formation is supplied from within by way of the tracheal system.

These oxidations take place only after the pupal contraction, although phenols and oxidase are present together earlier in the larval cuticle. In the larva the phenols are protected against enzymatic oxidation by the lack of oxidase from the procuticle, and from spontaneous oxidation by the presence of the impermeable epicuticle. It seems that oxidation of cuticular phenols in the procuticle is not possible, in the absence of oxidase, at the low oxygen tension prevailing there. It follows that the inward movement of oxidase into the procuticle is of paramount importance in promoting the oxidations which lead to tanning of the puparium.

This inward migration requires explanation. The fact that it takes place as the presumptive exocuticle of the white puparium becomes impregnated with sterol, but not when impregnation is prevented by removal of the epidermis, suggests that this substance may act as a vehicle for the oxidase. Further attention is drawn to the sterol by the observation that spontaneous oxidation after removal of the epicuticle takes place before impregnation but not after, an indication that the sterol is important in limiting the permeability of the procuticle. It is evident that this component of insect cuticles deserves further study.

ZUSAMMENFASSUNG

Die Entfernung der Epikutikula von Larven von *Calliphora vomitoria* bewirkt, daß die darunter liegende „Prokutikula" infolge Oxydation ihrer Dioxyphenole eine dunkle Färbung annimmt. Diese Oxydation erfolgt nicht enzymatisch. In der unverletzten Larve schützt die Epikutikula die Phenole vor spontaner Oxydation, so daß sie später bei der Bildung des Pupariums zur Verfügung stehen. Die Umwandlungen, welche während des Herannahens der Pupariumbildung in der „Prokutikula" stattfinden, werden beschrieben und ihre Bedeutung in Bezug auf den Beginn der Gerbung besprochen.

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RESPONSES OF *APHIS FABAE* SCOP. TO WATER SHORTAGE IN HOST PLANTS IN POTS

BY

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Water strain in the host plant reduced aphid feeding and larviposition and sometimes increased the proportion of winged offspring. These negative effects on the aphids occurred despite the enrichment of the phloem sap that could be inferred from the senescence and changing composition of the leaves under water strain, tended to obliterate the normal effects of leaf age, and were readily reversible. They are attributed to stimuli associated with a reduction in the quantity of sap obtainable by the aphids owing to reduced turgor pressure or increased sap viscosity. The opposite, positive effects recorded previously with various aphids may be associated with less severe or intermittent water strain in the plant when the reduced quantity of sap obtainable is more than compensated by its improved quality.

The literature is equivocal concerning aphids in relation to the water status of their host plants. The general impression of field observers has been that drought favours aphid multiplication on crops, leading sometimes to severe outbreaks (COTTIER, 1953; WALKER, 1954; WILSON, 1946). No single factor is likely to explain such complex field events, but there is little doubt that water strain in the plant can sometimes favour aphid multiplication. In the warm dry summer of 1949 when the leaves of sugar beets were undergoing precocious and accelerated senescence, there was little evidence of the "trough" in infestation by *A. fabae* on the middle-aged leaves which was noticeable in a wet season and on watered pot plants (KENNEDY, IBBOTSON & BOOTH, 1950). TAYLOR (1955) offered circumstantial field evidence that the drought-hastened senescence of potato foliage similarly favoured multiplication of *Myzus persicae* (Sulzer). In the greenhouse, MARKKULA (1953) showed experimentally that reducing the frequency and amount of watering of cabbage plants substantially increased larviposition by *Brevicoryne brassicae* L.

Such findings are consistent with the general experience of senescence and withering in herbaceous leaves when water is short (RUSSELL, 1950); with the plant-biochemical evidence that water shortage entrains hydrolytic changes in leaves, likely to enrich the translocatory phloem sap with breakdown products as in normal senescence (MOTHES, 1931; MAGNESS, DEGMAN & FURR, 1935; PETRIE & WOOD, 1938; CRAFTS, CURRIER & STOCKING, 1949; GATES, 1957); and with the aphidological evidence that normal senescence favours the settling (feeding), larviposition and growth of certain aphids including *A. fabae,* and is in fact accompanied by a rise in the concentration of nutritionally essential amino-nitrogen in the sap being obtained by from the phloem by feeding aphids (LINDEMANN, 1948; MITTLER, 1958a & b; KENNEDY, 1958).

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However, the larvae deposited on Markkula's water-deficient cabbages included a higher proportion of alatae than those deposited in smaller numbers on fully-watered plants. This seemingly paradoxical, "unfavourable" effect of water shortage in the plant has been recorded in a number of aphid species by workers concerned only with the problem of form determination (see LAL, 1952, 1955). That feeding and larviposition, too, could be reduced instead of being increased by water shortage in the plants, was first suspected during cage experiments on the effects of leaf age on *A. fabae* in warm dry weather in 1948 (KENNEDY & BOOTH, 1951), when it was also observed that the adults in free infestations on wilting sugar beets became very restless, many walking off the plants altogether (KENNEDY *et al.,* 1950). This suggested that the aphids' feeding rate might depend on the sap being under pressure in the plant, and so on plant turgor. Severing the rostra of feeding *A. fabae, Tuberolachnus salignus* (Gmelin) and *M. persicae* confirmed this as described elsewhere (KENNEDY & MITTLER, 1953; MITTLER, 1957; VAN SOEST & DE MEESTER-MANGER CATS, 1956). Meanwhile experiments were continued to confirm that water shortage could have unfavourable effects on the feeding, larviposition and form of *A. fabae,* and to throw light on the ecological consequences. The work on spindle (*Euonymus europaeus* L.) and sugar beet (*Beta vulgaris* L., var. Bush E) was done (by J.S.K. and C.O.B.) in the greenhouse and in the open soil in 1948—57, and that on broad beans (*Vicia faba* L., var. Seville Longpod) was done (by K.P.L.) in the greenhouse in 1953—54. This first paper deals with experiments on potted plants and the field work will be described elsewhere.

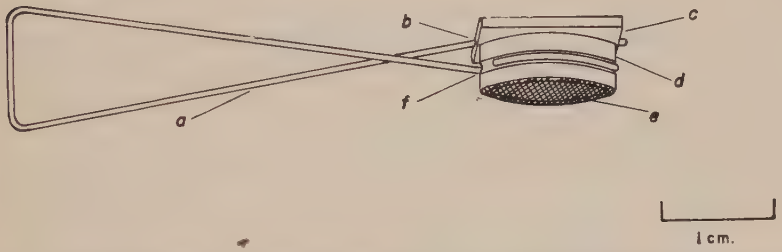

Fig. 1. Clip-on leaf cage for a single reproducing adult. *a.* stainless steel wire spring, sealed into the groove *b* in the 'Perspex' plate *c* which lies on top of the leaf, while the aphid compartment *d* (made of 'Tufnol' tubing and closed below with fine-mesh nylon stocking *e*) is held against the lower leaf surface by the other arm of the wire which clips into the groove *f*.

MATERIAL AND METHODS

Alate and apterous *A. fabae* from the greenhouse culture described by KENNEDY & BOOTH (1950) were used for all the cage experiments, the methods of aphid handling and the cages and procedure for the preference experiments being detailed in the same paper. All experiments except the first cited below were done in the greenhouse. The aphids were confined on broad bean leaves in the small clip-on cages shown in Fig. 1. Alatae were used for the preference experiments on spindle and apterae for the experiments on broad bean. Details of the plant material used were given by KENNEDY & BOOTH (1951), and the system for visual assessment of leaf age (employing various combinations of the symbols *Y* — young, growing; *M* — mature; and *O* — old, senescent) was set out in KENNEDY *et al.* (1950). Water shortage in the plants was usually brought about by simply ceasing to water the pot soil. Water and dry matter contents were estimated from discs punched out of the leaves with a cork borer, weighed at once and then dried to constant weight over-night in an oven at 103° C. Discs were punched midway between the midrib and the border of the leaf near its centre region in such a way as to include about the same proportion of vein in parallel and successive samples. The relative turgor of the leaves was judged from their external appearance — i.e. the extent to which they drooped. Some discs were taken from bean leaves for chemical analysis, the methods of sampling and analysis, together, with a consideration of statistical aspects of sampling and analysis, being described elsewhere (LAMB, 1957).

RESULTS

Effect of water shortage on leaves of similar age

1. *Free colonisation of Spindle outdoors*

Eight potted Spindle plants were taken which had been drastically pruned some weeks before and had then put out new shoots, but had not been watered further so that the growth was soon arrested. They were sorted into four pairs of like plants and one of each pair was watered daily (Lot A) while the other was left unwatered (Lot B). The A and B plants were set out alternately along a bench in the garden and their positions reversed daily for nine days of fine weather in June-July, 1949, when many *A. fabae* were on the wing. When the aphids infesting the plants were counted at the end of this period the unwatered B plants were flagging slightly while the A plants were erect and had put out new shoots. The aphids were then killed and washed off and the new shoots on lot A, and other minor shoots on both lots, were removed. From then on Lot B was watered daily and Lot A left unwatered for 21 days before the counts were repeated, during which time the B plants made considerable growth. Over 3 inches of rain fell during the 8th—11th days of this second period and few aphids were seen on the wing after the 7th day, while those on the plants came under heavy attack from Coccinellids and Syrphids and were entirely eliminated soon after the counts. Nevertheless, Table I shows that in both periods heavier aphid infestations had developed on the watered plants.

The aphids on the unwatered plants were not only fewer but also less aggregated, and the young were smaller in size. At the first counts when there had been relatively little predation it was noticed that motherless grouplets of young were more common on the unwatered plants, suggesting that more of the alatae depositing young had left again from these plants. The figures in Table I (final column) confirm that alate mothers were relatively even scarcer than young on the unwatered plants.

The heavier infestation of the watered plants was not due to preferential colonisation of new growth which only the watered plants produced. In the first period the alatae were counted and removed from all the plants on the first and third days before any appreciable new growth had appeared, and counted again without removal on the fifth day, in addition to the final counts shown in Table I. The numbers of alatae per 100 mature leaves on the old shoots, watered: unwatered, were 38 : 25, 25 : 12 and 63 : 37 respectively, on those days. In both periods aphids were extremely rare on the new growth even at the final counts. Thus the supply and deprivation of water produced reversible changes in the same, mature leaves.

TABLE I

Free colonisation of watered and unwatered Spindle plants in the field. Means and standard errors per plant from four plants of each lot

		No. of leaves	Alate mothers per leaf	Progeny per leaf	Progeny per mother present
First period	Lot A watered	66	1.7 ± 0.3	18.5 ± 1.6	11.0
	Lot B unwatered	51	0.5 ± 0.2	8.0 ± 2.7	15.0
Second period	Lot A unwatered	27	0.2 ± 0.1	0.7 ± 0.5	3.8
	Lot B watered	24	1.9 ± 0.8	5.5 ± 1.0	3.0

2. *Feeding preferences on Spindle*

Four similar Spindle plants were selected from a batch which had been kept well watered in the greenhouse and bore long shoots in active growth. Eleven pairs (in general, Spindle shoots bear leaves in decussate pairs) of mature, middle leaves were selected from among those on the main shoots of two of the plants (Lot A), to match as closely as possible eleven pairs of leaves on the two other plants (Lot B), in respect of appearance and position on the shoots. Watering of Lot B was now stopped while daily watering of Lot A was continued. On the 11th day the soft growing shoot tips of Lot B drooped at midday and plant B. 1. which was badly affected was watered. The B plants were not watered again until the evening of the 19th day. By the 18th day the shoot tips of the B plants were again flaccid, while the mature and older leaves although not

TABLE II

Mean water and dry matter contents of 11 discs (8.7 mm. diam.) taken from the same mature leaves on two lots of Spindle plants, just before and 6 days after reversal of the watering regime

		Water mg.	Dry matter mg.	% water fresh weight
First period	Lot A watered	10.76	3.55	75.1
	Lot B unwatered	8.83	3.20	73.4
Second period	Lot A unwatered	10.24	3.33	75.1
	Lot B watered	9.95	3.16	75.9

visibly wilting had become distinctly paler than the A leaves. On this day the "matched" leaves of A and B were caged together in two-leaf cages to test the feeding preferences of alate aphids. At the same time a disc was punched out of the leaf opposite on the stem to each caged leaf for water and dry matter contents (Tables II and III). The preference tests were repeated with the same leaves on the 19th day. Thereafter the B plants were watered daily and the A left unwatered. Six days later the A plants were beginning to droop at the shoot tips and their leaves had paled (although they were still a darker green than the B leaves which had only partially regained their colour), while the B shoot tips were by now fully erect. Aphid preference testing and disc-sampling were then repeated on the same leaves as before.

TABLE III

Same data as for Table II analysed to show mean differences between the "matched" leaves of Lot A and B at each sampling, and mean changes in the leaves of each lot between samplings (with standard errors)

		Water mg.	Dry matter mg.
Difference between matched leaves, A *minus* B	First sampling	+1.88 ± 0.31	+0.35 ± 0.07
	Second sampling	—0.13 ± 0.28	—0.15 ± 0.11
Change between samplings, 1st *minus* 2nd	Lot A	—0.64 ± 0.17	—0.25 ± 0.10
	Lot B	+1.17 ± 0.22	—0.05 ± 0.07

A majority of the alatae settled to feed on the leaf attached to the watered plant (Lot A on the first occasion, Lot B on the second), with a minority on the matched (unwatered) leaf, in all cages on both days on both occasions: i.e. 22 tests before and 22 after reversal of the watering regime. It should be emphasized that the two-leaf cage is not a sensitive method of demonstrating preferences (KENNEDY & BOOTH, 1950), the apparent strengths of preference (majorities) obtained in such cages being considerably less than those observed when free aphids were presented with a similar choice in the field (above). All the discs showed a loss of water when watering was suspended. Note in Tables II and III the loss shown by B compared with A at the first sampling, and the loss in A between the two samplings. When the tips were turgid again after watering had been resumed (Lot B, Table III) every disc now showed a again of water. In other respects the leaf discs did not show consistent differences related to the aphids' preferences.

3. *Feeding and larviposition on Broad Bean*

Twelve plants were kept normally watered up to the three-node stage, after which six were left unwatered while regular watering of the other six was continued. Eight days from the start of this regime, two apterous adult were caged individually on the youngest unfurled leaf of each plant and their larviposition recorded for seven days. The aphids on the unwatered plants were more restless and produced less honeydew than those on the watered plants. On the second day for example half the aphids caged on the unwatered plants were not feeding while those on the watered plants were all feeding. The mean daily larviposition rate was consistently higher on the watered plants, although it gradually declined on both sets of plants. After two days the mean number of larvae per parent was

8.2 on the watered and 4.3 on the unwatered plants; after seven days it was 20.1 and 10.5 respectively, the difference being highly significant.

Striking effects of water supply on honeydew production were observed subsequently in *Brevicoryne brassicae* L. on Swede plants (*Brassica rapa* L.) (Cp. MITTLER, 1957, on cessation of sap exudation from cut stylets of *Tuberolachnus salignus* (Gmelin) on willow stems denied water). When the Swedes had been left unwatered for several days honeydew production had diminished to the point of virtual cessation, but it began again with most of the individuals producing large droplets within 30 minutes of watering the plants.

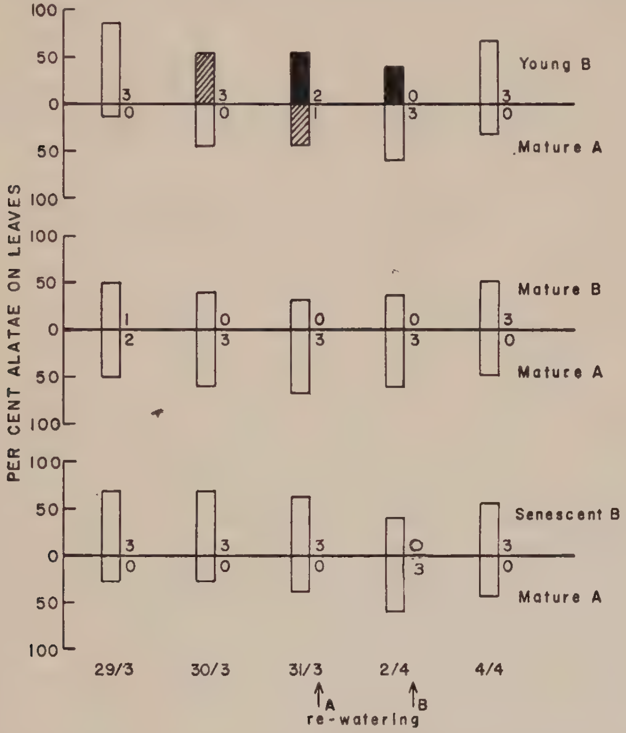

Fig. 2 Feeding preferences of alatae between Spindle leaves of 3 ages on one lot of plants (B) and mature leaves on another lot (A). Each vertical block indicates the percentage distribution of feeding aphids between the two leaf ages stated, as a mean for 3 cages with 30 aphids per cage. Figures beside the blocks show the number of cages in which a majority of the aphids settled to feed on each leaf. Shading indicates partial or complete wilting.

Effects of water shortage on leaves of different ages

1. Feeding preferences on Spindle

(I) Four actively growing plants were used, two of which (Lot A) provided nine mature leaves for enclosure in 2-leaf cages with three growing, three mature and three senescent leaves on the other two plants (Lot B). Both lots had been denied water for several days before the preference tests began. When both lots were still fully erect (Fig. 2, 29/3) the young and senescent leaves of Lot B were preferred to the mature leaves of Lot A, and the mature leaves of the two lots were fed upon about equally, as expected from previous work. But as the growing shoot tips of Lot B began to droop on 30/3, Lot A being still erect, the distribution of settled aphids shifted over toward Lot A. This shift went further when Lot B wilted badly on 31/3 and Lot A were beginning to droop; and went further still on 2/4 after watering and recovery of shoot tip turgor in Lot A. The mature A leaves were then preferred to all ages of leaf on Lot B which was still unwatered and drooping badly. But on 4/4, after watering and recovery of Lot B as well, the young and senescent B leaves regained their preferred status over the mature A leaves.

TABLE IV

Mean percentages of alatae settled on three ages of leaf on one lot of Spindle plants (B) when these were all caged alongside mature leaves of a second lot of similar plants (A). Figures in brackets show the number of tests in which a majority of aphids in the cage settled on the B leaf

Age of B leaf	Both lots unwatered	A only re-watered	Both lots re-watered
Young	67 (9)	40 (0)	66 (6)
Mature	56 (7)	41 (0)	54 (4)
Senescent	61 (9)	42 (0)	57 (6)
Total tests	9	6	6

(II) The experiment was repeated with the same number of leaves on fresh plants. Three sets of preference tests were done when neither lot of plants had been watered for some days; two sets were done on the next day when the A plants had recovered their turgor after watering the previous evening, but Lot B were now more severely wilted at the tips than A had been the previous day; and two final sets of tests were done two days later when both A and B were fully erect, B as well as A having been watered the previous evening. In the early stages of this experiment, unlike the previous one, the A plants wilted in advance of the B. Correspondingly, Table IV shows that even the mature B leaves were preferred to those of A until A had been watered. Otherwise the results reproduced those of the last experiment. Discs taken from the leaves opposite on the stems to the three sets of matched mature leaves again showed changes of water content and presumably of turgor in the leaves corresponding to the reversing preferences of the aphids, while in other respects the discs did not show correlated differences.

TABLE V

Mean number of larvae deposited per aphid, by 3 apterae in 3 days on 3 ages of leaf on similar Bean plants: watered, and unwatered in an air current

Age of leaf	Watered	Unwatered
Young	14.7	5.7
Mature	5.0	2.3
Old mature	2.3	1.0

Least significant difference at 5% = 4.7; at 1% = 6.6.

2. *Larviposition on Broad Bean*

A 5-node plant was wilted in a current of air without watering for 4 days. Wilting was most severe in the oldest leaves and progressively less apparent up the plant. The youngest leaves remained normally erect throughout. Senescence (visible yellowing) was observed only in the oldest leaves as wilting proceeded. These leaves became flaccid, then passed rapidly into an irreversibly wilted state and withered before much yellowing had appeared. 26 hours after the start of the experiment 3 clip-on cages were fitted to young, mature and old mature ($M(O)$) leaves of this plant and to comparable leaves of a normally watered plant close by but not in the air current. An apterous adult aphid was placed in each cage and the number of larvae born was recorded 72 hours later. There was both a leaf-age effect and a water treatment effect on larviposition (Table V), and the leaf age differences were reduced by water deprivation.

TABLE VI

Mean number of larvae deposited per aphid, by 6 apterae in 2 days on 2 ages of leaf on similar Bean plants: Lot A watered throughout; Lot B left unwatered for 5 days, then re-watered

Age of leaf	Lot B unwatered		Lot B re-watered	
	A	B	A	B
Young	1.19	0.74	1.24	1.11
Mature	1.18	0.36	0.57	0.16

3. *Larviposition and form of offspring on Broad Bean*

(I) Of 12 similar potted plants, 6 were left unwatered for 5 days and then re-watered (Lot B), while the other 6 were kept normally watered throughout (Lot A). Three days after the start of this treatment an apterous adult aphid was caged on the youngest unfurled leaf and on the third oldest leaf of each plant, and the number of larvae counted at 48 hours. The day after re-watering of Lot B a further batch of aphids were caged on the same leaves for two days' larviposition. The larvae were subsequently classified into apterae and alatae, and the results are summarized in Table VI. Larviposition was significantly reduced on the unwatered (B) plants ($P = 0.05$) compared with the watered (A) plants irrespective of leaf age. After rewatering of Lot B the larviposition rate was not significantly different between the two lots. Nor did it differ significantly between the young and mature leaves on either lot of plants at the first test. It was higher ($P = 0.01$) on the young leaves at the second test. A significantly higher proportion of alatae were produced on mature than on young leaves on both lots in both tests, with no significant effect of water supply on form.

(II) The next experiment was a randomized block experiment carried out at a mean temperature of 10 ± 4° C. and day lengths of 8.2—10.2 hours: conditions likely to produce autumn forms of the aphid (DE FLUITER, 1950). When the alate progeny born during the experiment eventually became adult they proved most reluctant to feed on Bean but fed readily on Spindle, and all their progeny on either host were oviparae. These alatae were thus gynoparae, not virginoparae as in the other experiments; and this was checked by counts of the sensoria on the fourth segment of the antennae (KENNEDY & BOOTH, 1954). Over two thirds of specimens bore 2 or more sensoria on the segment, whereas over two thirds of specimens from the warm 16-hour-day culture bore one sensorium or none.

TABLE VII

Mean larviposition over first 3 days and survival time of 4 apterae on 3 ages of leaf on Bean plants kept on 3 watering regimes, A — high, B — medium and C — low (see text); together with mean percentages of unborn embryos found mature at the mother's death, and of alate young born

	Age of leaf	Watering regime		
		A	B	C
Larvae per mother	Young	8.0	5.8	5.5
	Mature	5.8	5.8	5.3
	Old mature	6.5	5.0	4.8
Survival of mother, days	Young	13	23	6
	Mature	15	10	7
	Old mature	8	15	9
			(least sig. diff. at 5% = 6.6)	
Mature embryos per cent (as equivalent angles)	Young	29	26	43
	Mature	28	31	34
	Old mature	26	41	55
			(least sig. diff. at 5% = 10)	
Alate young per cent	Young	30	23	71
	Mature	21	72	83
	Old mature	15	67	100

Four plants were maintained at each of 3 water levels: A — watered to field capacity daily; B — watered whenever the lowest leaves began to wilt; C — given only enough water for survival, being more or less wilted throughout. Lot B received twice as much water as Lot C, and Lot A 2½ times as much as B, over the whole period of 37 days. On the first day the treatments began, an apterous adult aphid was caged on the youngest unfurled leaf, a middle mature leaf and the oldest mature leaf on each plant. As the parent aphids died they were dissected and unborn embryos counted and measured. The results are summarized in Table VII. The larviposition rate, recorded only during the first 3 days of this experiment, was then insignificantly higher on the younger leaves and better-watered plants. But the mothers on the badly-wilted C plants died off sooner than those on the other lots ($P < 0.05$ for the difference between the combined means of C and B), retaining a larger proportion of mature embryos ($P < 0.05$ for the difference between the combined means of C and A or B) indicating an inhibition of parturition, and the larvae they had deposited included more alates ($P < 0.05$ for the difference between the combined means of C and A). In these three respects the aphids on the medium water regime B plants did not fare significantly worse than those on the continuously turgid A plants, except for the production of more alate young on the mature and old-mature leaves where the slight and intermittent wilting occurred. On the young leaves, they fared insignificantly but consistently better on B than A. There was also an effect of leaf age on form, in that significantly fewer alates were produced on the youngest than on the older leaves of lots B and C (but not of A, on the young leaves of which most crowding occurred).

4. *Leaf Composition*

Sugar Beet. Water and dry matter in leaf discs (1 per leaf at each sampling) taken at intervals from a well-grown vegetative sugar beet which was denied water for 8 days and then watered again, are shown in Fig. 3. As with Beans wilting was most severe in the oldest leaves and was progressively less apparent

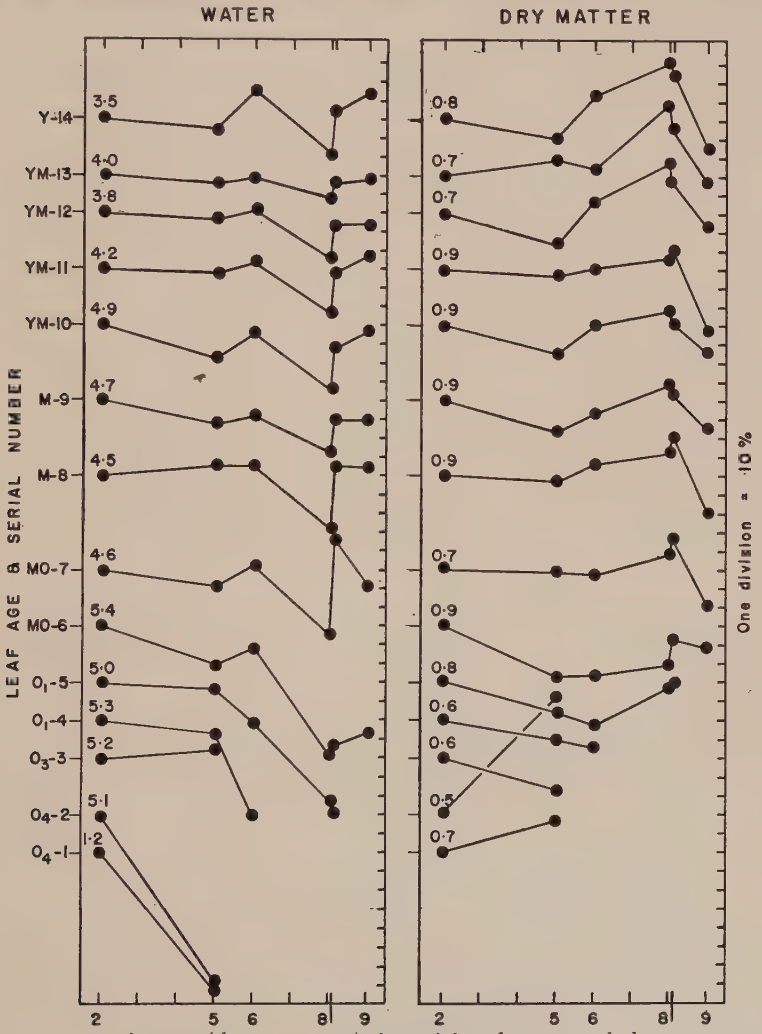

Fig. 3. Percentage changes (day 2 = 100) in weight of water and dry matter per disc (5.3 mm. diam.) taken from all the leaves of a Sugar Beet plant left unwatered until immediately after the first reading on the 8th day. Figures at left end of each curve show the initial absolute weights in mg per disc.

toward the youngest. The oldest leaves, Nos. 1—4, died off before watering was resumed; No. 5 failed to recover turgor the day after re-watering and Nos. 6 and 7 recovered only partially. Nos. 8—14 did not wilt markedly until the 8th day and had recovered full turgor 2½ hr. after watering.

During the days of water deprivation, senescence was proceeding in the *M* and older leaves (1—9) more rapidly than in similar plants that were kept fully watered alongside. There were indications of a corresponding loss of dry matter (presumably by outward translocation) in most leaves at first (the gain in leaves 1 and 2 was associated with severe shrinkage), as observed in Spindle (Tables II and III). But later, all the surviving leaves 5—14 showed a gain of dry matter, which in the older leaves continued during the first 2½ hr. when water was returning to the leaves; and finally, 20 hr. after watering all the leaves showed a loss of dry matter to a level below that at the beginning of the experiment, a loss which in the young leaves began during the first 2½ hr. and could have been due at least in part to growth expansion of the lamina. That this final major fall in dry matter content per disc represented a genuine net loss in the mature and older leaves and was not simply due to lamina expansion with recovery of turgor, is indicated by the persisting high level of dry matter in the discs 2½ hr. after watering when turgor had already been regained, the major fall following this. The loss of dry matter appeared rather to be due to a "surge" of outward translocation of hydrolytic products from the leaves after an arrest of translocation in the later stages of water deprivation.

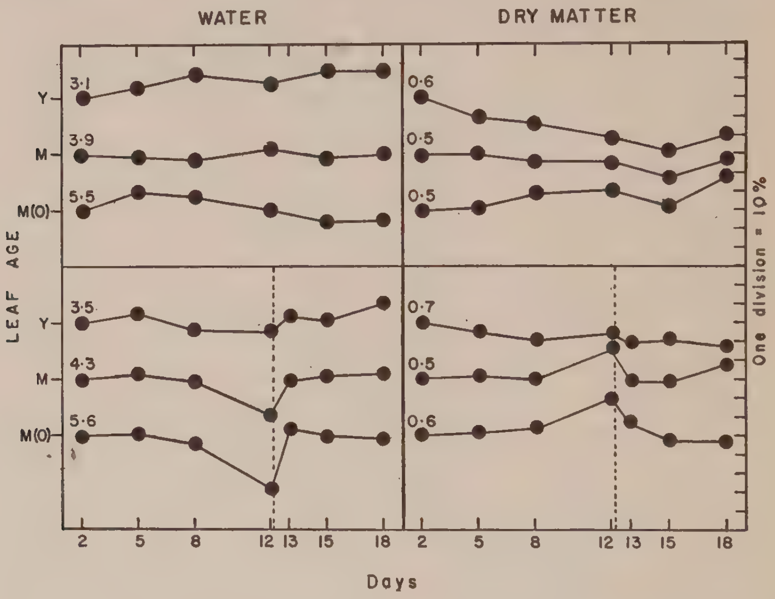

Fig. 4. Percentage changes (day 2 = 100) in mean weight of water and dry matter per disc in duplicate discs (5 mm. diam.) taken from 3 ages of leaf on a continuously-watered Broad Bean plant (above), and on a similar plant (below) left unwatered until immediately after the reading on the 12th day. Figures at left of each curve, as in Fig. 3.

A similar loss of dry matter after resumption of watering was shown in all the 14 leaves of another vegetative beet which was denied water only long enough for the oldest leaves (1—5) to wilt, disc-sampled, then watered and sampled again 20 hr. later. A third vegetative beet with 18 leaves including 6 *M* was kept unwatered in the shade of the laboratory to retard water loss and confirm the loss of dry matter during this process, before re-watering. By the 6th day the 7 oldest leaves had wilted (3 died) but 13 of the surviving 15 leaves showed a loss of disc dry matter since the 2nd day.

Broad Bean. Water and dry matter in duplicate discs taken at intervals over 18 days from the leaves of two similar plants, one of which was watered daily while the other was denied water for 12 days and then watered again, are shown in Fig. 4. The same young, mature and old-mature leaves on each plant were sampled throughout. On the 8th day the four oldest leaves on the unwatered plant were slightly flaccid. On the 12th they were definitely wilted, and after that

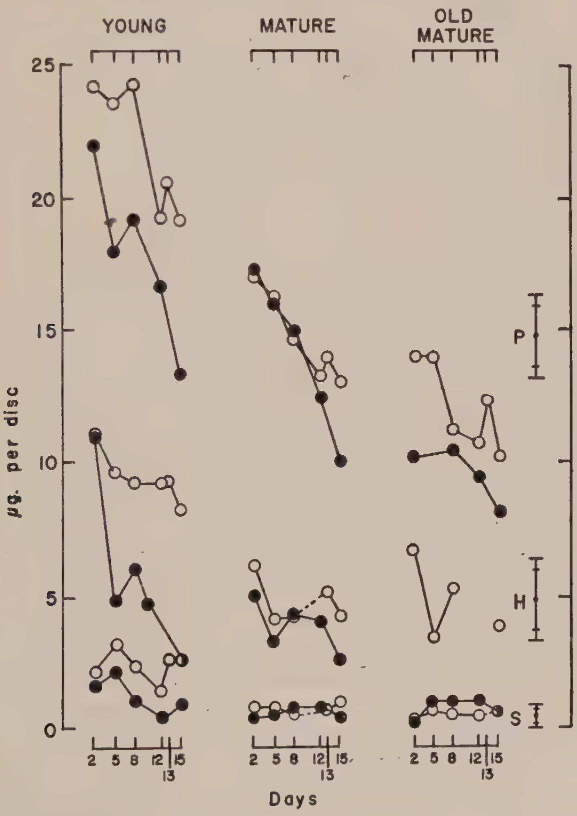

Fig. 5. Mean weights per disc of three amino-nitrogen fractions in the same leaves as Fig. 4: protein (alcohol-soluble) fraction — P; "asparagine" (hydrolysed alcohol-soluble) — H; and unhydrolysed alcohol-soluble — S. Least differences for significance at 1% and 5% levels shown on right. Solid dots — continuously-watered plant; open circles — plant left unwatered until 12th day.

day's disc-sampling this plant was watered and by the following day all its leaves had recovered their turgor. In contrast to the Spindle and Beet leaves, little senescence was visible and there was no indication of loss of dry matter in the mature and older leaves as a result of the water deprivation. But the disc dry matter increased slightly in the wilting plant on the 12th day, then decreased again after watering, as in the Beets, and the increase was again unlikely to be due simply to lamina shrinkage since no increase in protein nitrogen accompanied it.

Changes in the amino-nitrogen fractions in the same discs are shown in Fig. 5.

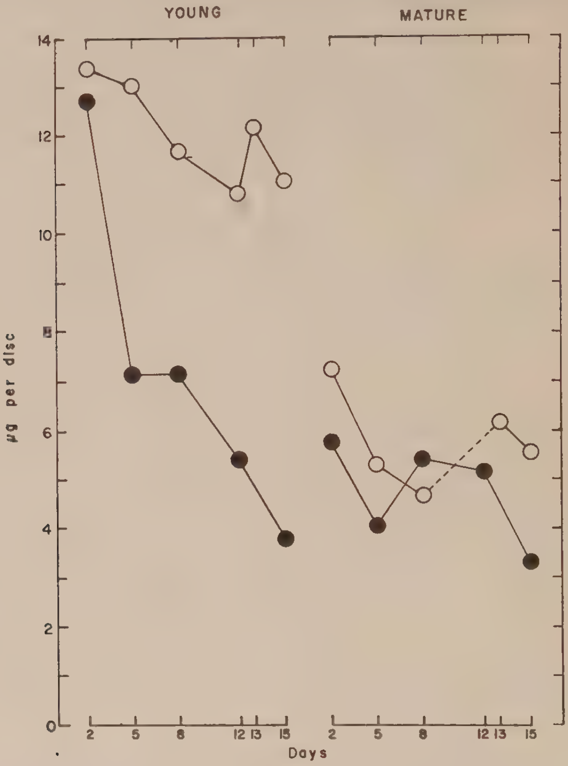

Fig. 6. Summed "S" and "H" fractions from Fig. 5.

The most striking effect of water deprivation was the maintenance at a high level in the young leaves of the fraction which appeared on hydrolysis of the alcohol extracts ("H" in Fig. 5) and is referred to below as "asparagine-nitrogen" although alcohol-soluble protein or peptide could have contributed to it. When watering was resumed there was a temporary increase in residual (protein) nitrogen ("P") in all leaves, and in the young leaves there was a persistent increase in amino-nitrogen in the unhydrolysed extracts ("S"). Since organic nitrogen is directly available to phloem-feeding aphids only in its soluble forms in the sap, the fraction of most aphidological interest is the total non-protein fraction ("H" *plus* "S"), shown in Fig. 6. This remained at a much higher level in the young leaves of the unwatered plant from 7 days before until at least 3 days after watering was resumed.

DISCUSSION

The results incidentally conform for Broad Bean that the larviposition rate of *A. fabae* tends to be higher on growing than mature leaves as with other plants previously tested; and confirm also that fewer alate offspring tend to be produced on the young leaves (Cp. JOHNSON, 1958). The second peak of larviposition recorded on senescent Spindle and Sugar Beet leaves was not found on Broad Beans, and in general, we have observed only slight indications of such a second phase of suitability in Bean leaves (e.g. TAMBS-LYCHE & KENNEDY, 1958). Slow progressive senescence with loss of day matter as in Spindle and Beet (where rapid senescence was found less suitable for the aphids: IBBOTSON & KENNEDY, 1950) does not seem to occur in Bean leaves under similar conditions. Senescence of Bean leaves proceeds rapidly once started and is often, especially under water strain, preceded or cut short by withering.

It is known from the work on other aphids cited in the Introduction that the phloem sap is richer in amino-nitrogen during the growth and senescence of foliage than during its intermediate mature stage, and the as yet unidentified stimuli to which the aphids respond by feeding and larvipositing more, and producing fewer alate offspring, appear to be associated in some way with this enrichment of their food (KENNEDY, 1958). No information is available on the composition of phloem sap in Beans (or any herb) but the lamina of the young Bean leaves was found to certain more soluble amino-nitrogen than that of mature leaves.

But the reduction of feeding and larviposition and the production of more alates on water-deficient plants, recorded in the present experiments, cannot be interpreted on the same lines. On the contrary, the young leaves of the water-deficient Beans contained more, not less, amino-nitrogen than those on the turgid plants. In older leaves of Spindle, Beet and Bean, the water deprivation induced varying degrees of visible senescence, with detectable dry matter losses in Spindle and Beet, from which again some enrichment of the phloem sap would be inferred during the outward translocation of breakdown products. Yet water deficiency reduced feeding on leaves of all ages, masking the differences between them and rendering previously preferred growing and senescing ones less acceptable than turgid mature ones. Unlike the leaf age effects in turgid plants, the unfavourable effects of water deprivation were readily reversible. Quite a different mechanism must, it appears, be postulated for them. We infer that they are responses to some stimuli associated with the reduction in the rate of flow of sap up the aphids stylet canal, which was observed by MITTLER (1957) from severed stylets of *T. salignus* in water-deficient willow bark. This would result from the fall in hydrostatic pressure or the increased viscosity of the cell sap, when water is lost.

The contradictory reports in the literature could then be reconciled on the assumption that the effects of water strain in the plant are in fact contradictory for the aphids: reducing the quantity of sap they can get but at the same time improving its quality. For the relative magnitude of these two opposite effects certainly varies. Variations between different kinds of plant in the same conditions will be the subject of another paper, but reference has already been made to the earlier cutting short of senescence by water loss in Bean than in Beet, and differences between woody and herbaceous plants are touched upon by KENNEDY & BOOTH (1958; also KENNEDY, 1958). Here we may consider briefly variations in the same kind of herbaceous plant under different conditions.

In the present experiments (with one exception, see below) the water strain was intentionally brought on quickly, or allowed to develop through soil drought for some days before the aphids' responses were tested, visible wilting was then in progress, and the aphids' responses were consistently negative. In MARKKULA's (1953) experiments on *B. brassicae* temperatures were rather low, the aphid testing was started sooner after watering was stopped, before wilting or senescence were visible, and water was re-supplied at least once during the experiment. The effect on larviposition was positive, and recalls the preferential colonisation by *A. fabae* of turgid Spindle and Beet leaves at the stage of "invisible senescence" (KENNEDY *et al.*, 1950; IBBOTSON & KENNEDY, 1950). When as mentioned in the Introduction *A. fabae* adults walked off outdoor Sugar Beets in 1948, severe wilting had been brought on by a " heat-wave" in an otherwise wet year; when an opposite, favourable effect was observed in 1949, the water strain had developed more gradually. Water strain usually develops more or less gradually and intermittently in the field so that the effect on aphids on herbs may usually be favourable, with the eventual unfavourable effect when water strain becomes acute coming too late to have any economic interest. So long as the daily loss of turgor is not too great or prolonged there may be a net gain for the aphids, through the sap enrichment affecting their nutrition and some associated stimuli affecting their behaviour. The greater the temporary water deficit, up to a point, the greater may be their gain every time the plants recover, at night or with a break in the weather, and there follows a "surge" of outward translocation of breakdown products through the phloem.

In various other aphid species (see BONNEMAISON, 1951) it has been shown that a short period of starvation provides an adequate "signal" stimulus for wing-determination in the offspring, and in *Rhopalosiphum pseudobrassicae* (Davis) (= *Lipaphis erysimi* Kltb.). LAL (1952) found that 6 hours' starvation was more effective in this respect than longer periods. We might then expect to find an intermediate stage in the development of plant water strain, when the enrichment of the sap was still ensuring the rapid production of large vigorous aphids, while at the same time the intervals of impeded sap uptake (aided in some cases by crowding) were turning a growing proportion of them into alates. The latter would serve to spread the infestation in the field, and the combination of the two effects would seem ideal for an outbreak. Just this point of balance between the positive and negative effects appears to have been struck by MARKKULA (1953) in his work on *B. brassicae*, while BONNEMAISON (1951) missed it, using the same aphid and plant but a different watering regime, and obtaining large apterae (uncounted) but very few alates. Markkula's combination was perhaps approached, but not reached, with treatment B in the last Bean experiment above. It should be profitable to make a systematic study of the changing balance of effect on different aphids on different crop plants as water strain develops at different rates.

ZUSAMMENFASSUNG

1. Die Wirkungen beträchtlichen Wassermangels getopfter Wirtspflanzen auf Ansiedlung und Vermehrung von *Aphis fabae* Scop. wurde in Gewächshausversuchen mit im Labor herangezogenen geflügelten Virginoparen an *Evonymus europaeus* L. und mit Ungeflügelten an *Vicia faba* L. sowie in einem Feldversuch bei der natürlichen Besiedlung von *E. europaeus* untersucht.

2. Der Wassermangel verminderte Ansiedlung und Larvenproduktion und vergrößerte den Anteil geflügelter Nachkommen. Diese negativen Wirkungen können die normalen Wirkungen des Blattalters auslöschen, waren aber durch Wiederversorgung der Topferde mit Wasser leicht reversibel. Sie traten trotz der Bereicherung des Phloemsaftes ein, die aus dem Altern und der veränderten Zusammensetzung der Blätter bei Wasserverknappung zu schließen war. Sie werden auf Reize zurückgeführt, die auf einer Reduktion der für die Blattläuse zur Verfügung stehenden Saftmenge beruhen, welche als Folge reduzierten Turgordruckes oder steigender Saftviskosität entsteht.

3. Die gegenteiligen günstigen Auswirkungen des Wassermangels, über die früher bei verschiedenen Blattläusen berichtet wurde, können auf weniger starkem oder vorübergehendem Wassermangel in der Pflanze beruhen, wenn die Verringerung der erreichbaren Saftmenge von ihrer erhöhten Qualität mehr als ausgeglichen wird.

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MULTIPLICATION OF POTATO LEAF ROLL VIRUS IN THE APHID *MYZUS PERSICAE* (SULZ.)

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Insect-to-insect transmission of potato leaf roll virus proved possible by injecting either juice or haemolymph from viruliferous *Myzus persicae* into virus-free ones.

In serial transmission experiments, in which injection of haemolymph was practised, the virus could be carried through as many as 15 successive aphid-to-aphid passages without access of inoculated insects to any plants that are susceptible to leaf roll. The theoretical dilution of original virus used for the inoculation in the 15th passage would have been approximately 10^{-21}. It is concluded that such persistence of virus in the aphids could only be explained on the assumption that the insect itself sustains reproduction of the virus.

FUKUSHI (1935; 1939) was the first to show that the virus which causes rice-stunt is reproduced not only in the rice plant but also in its insect vector. Since then a number of authors have obtained evidence for the multiplication of other plant viruses in their insect vectors. The work done in this field up to 1955 has been extensively reviewed by MARAMOROSCH (1956a). It can be stated now with reasonable certainty that reproduction of plant viruses takes place in the following insect species: *Nephotettix apicalis* Motsch. var. *cincticeps* Uhl. (rice-stunt virus; FUKUSHI, 1935; 1939); *Macrosteles fascifrons* Stal. (aster yellows virus; KUNKEL, 1937; BLACK, 1941; MARAMOROSCH, 1950; 1952; 1955; 1956b); *Agalliopsis novella* Say (clover club-leaf virus; BLACK, 1950); *Agallia constricta* Van Duzee (wound-tumor virus; BLACK & BRAKKE, 1952).

The insect species mentioned all belong to the family Cicadellidae (Jassidae: Leaf-hoppers). Their transmission of virus is characterized by (a.) a relatively long incubation (= latent) period of the virus in the insect and (b.) a strong retention of the virus by the insect. This means that it takes a relatively long time for the insect to become infectious once it has had access to a source of virus (diseased plant), the virus then being retained by the insect for its whole lifetime.

This type of virus transmission is not restricted to Cicadellids; it is also encountered, for example, in Aphids (WATSON & ROBERTS, 1939; DAY, 1955a). There seems to be, therefore, no apparent reason to assume that the phenomenon of reproduction of plant viruses in their insect vectors occurs only in Cicadellid leafhoppers.

DAY (1955b), when studying the mechanism of transmission of potato leaf roll virus by the green peach aphid *Myzus persicae* (Sulz.), obtained the first evidence for the occurrence of plant virus multiplication in an aphid. This evidence was necessarily inconclusive since, as the author stated, insect-to-insect transmission of the virus by means of injection, which proved to be such an excellent tool in the study of virus reproduction in Cicadellidae (BLACK, 1941), too infrequently met with success to permit the technique to be used for passaging the virus.

In contrast with this statement, HEINZE (1955) reported far more frequent successful transmission of leaf roll virus by injection. In preliminary experiments carried out by one of the present authors, this was confirmed. It was, therefore, decided to attempt the performance of serial passage experiments in which leaf roll virus was to be transmitted mechanically from originally viruliferous *M. persicae* to successive groups of virus-free insects. Successful passage through a large number of originally virus-free aphids would be a strong indication for the occurrence of multiplication of the virus in the insects, as otherwise one has to assume that the original amount of virus could be diluted indefinitely.

MATERIALS AND METHODS

The *Myzus persicae* used throughout this study were maintained in the insectary of the Institute for Phytopathological Research (I.P.O.), Wageningen. Colonies of virus-free insects were reared on *Nicotiana glauca* L. Viruliferous aphids were obtained from cultures on *Physalis floridana* Rydb., infected with a strain of potato leaf roll virus, kindly supplied by Dr. BEEMSTER from the I.P.O. In all experiments adult apterous females were used.

Physalis floridana was also used as a test plant for potato leaf roll virus (KIRKPATRICK, 1948; MACCARTHY, 1954). Seeds were germinated on moist filter paper in petri dishes at approx. 27° C., and planted to flats with steamed soil as soon as the cotyledons developed. From then on the plants were maintained in the greenhouse at a temperature varying between 18° and 26° C. Transplanting to 4-in. pots was carried out after the appearance of the first leaf; the plants were used for infectivity tests when 2—4 leaves had developed. Usually clear symptoms would develop in about 10 days; generally, however, the plants were kept for 3 weeks in a separate greenhouse, which was fumigated at regular intervals with lindane. During the winter the test plants received additional light; nevertheless development of symptoms took about 8 days more. Accordingly, in winter time the plants were kept under observation for at least 4 weeks.

Injections were performed by means of the technique developed by WORST (1954). The apparatus used essentially consists of a fine, thickwalled capillary tube, one end of which is made into a micro-pipette with an outside diameter of 20—30 μ, the other end being sealed. The whole tube is filled with water and can be heated by means of an electric device. Thermal expansion of the water provides the force needed for injection. Upon cooling the water contracts again and thus fluid can be drawn into the injection needle.

Inoculations were carried out under the binocular microscope. The aphids were anaesthesized with gaseous carbon dioxide and handled by means of a vacuum system, consisting of a capillary with a bore just fitting an aphid's head and connected to a suction pump. The injection system was kept fixed; the insects were simply pressed onto the needle and injected intra-abdominally. Care was taken to insert the needle quite superficially into one of the intersegmental membranes on the dorsal side of the insect, a little out of the median. In this way about 30 insects could be inoculated per hour.

Additional details concerning methods are to be found in the text.

RESULTS

Insect-to-insect transmission. Preliminary experiments demonstrated that insect-to-insect transmission of potato leaf roll virus could be performed successfully by means of injection of either juice or haemolymph from viruliferous aphids into virus-free ones.

Insect juice was obtained by grinding a number of animals in a small agate mortar with an appropriate volume of neutral buffered salt solution (BLACK & BRAKKE, 1952) and centrifuging the resulting brei for 20 minutes at 4,000 r.p.m. in the cold. Haemolymph was obtained by the reversal of the injection procedure. The inoculated aphids were caged on *Physalis* test plants and transferred to fresh plants every three or four days, either in groups or individually.

TABLE I.

Transmission of potato leaf roll virus by injected Myzus persicae.

Virusfree aphids injected with approx. 0,004 μl of virus-containing solution and subsequently transferred for periods of 3—4 days to successive *Physalis floridana* test plants.

	Plants infected/total tested				
Test feeding in days after injection.	0—3	4—6	7—10	11—13	14—17
Aphids injected with					
Juice 1 : 100[a]	0/9	0/5	0/3	0/3	0/2
Juice 1 : 10[a]	0/8	0/8	4/8	3/8	2/4
Juice 1 : 10[b]	0/30	0/20	7/13	6/8	4/8
Juice 1 : 5[a]	5/10	2/9	1/5	—	—
Haemolymph	4/10	2/5	0/2	—	—
Average mortality[c] %	45	62	73	82	100
Non-inoculated-controls					
Viruliferous	20/20	17/20	16/17	—	—
Virus-free	0/20	0/20	0/20	0/20	—
Average mortality %	20	42	53	70	96
Insect-free control-plants	0/25				

[a] 5 aphids per test plant.

[b] 1 aphid per test plant.

[c] calculated from the data for insects injected with juice 1 : 100 and 1 : 10, since in the other experiments extremely high temperatures caused abnormal mortality in the second week.

The results of these experiments are presented in Table I. Apart from demonstrating the possibility of transmitting the virus by means of injection, this table shows the remarkable fact that upon injection of solutions with a relatively high virus content (1 : 5 diluted juice; haemolymph) the insect became more rapidly infective than when less concentrated solutions were inoculated. So there seems to be a relation between the duration of the incubation period and the amount of virus injected.

A more precise determination of this incubation period following the injection of approx. 0.003 μl of haemolymph from viruliferous into virus-free aphids gave results that are summarized in Table II, together with figures obtained for the incubation period following a "natural" acquisition feeding for 6 hours on leaf-roll-infected *Physalis*. These data suggest that the amount of virus injected was less than that which was ingested during the 6 hours' feeding period on the diseased plants.

TABLE II.

Incubation period of potato leaf roll virus in Myzus persicae.

Normally infected aphids allowed to feed for 6 hrs. on leaf roll diseased *Physalis floridana*. Injected aphids received approx. 0.003 μl of haemolymph from viruliferous aphids. The aphids transferred at regular intervals to fresh test plants.

Time after infection[a]	Plants infected/total tested	
hours	Normally infected aphids[b]	Injected aphids[c]
12	—	0/18
16	0/5	—
24	3/5	0/18
36	5/5	3/16
48	5/5	—
60	—	5/7

a Included 6 hrs. feeding on diseased *Physalis floridana*.
b 5 aphids per test plant
c 1 aphid per test plant

Next an attempt was made to establish the dilution end-point of virus present in the haemolymph of viruliferous aphids. Undiluted haemolymph and haemolymph diluted 1 : 10, 1 : 100, 1 : 1.000 and 1 : 10.000 with buffered salt solution were injected into groups of virus-free aphids, which were subsequently caged individually on *Physalis* test plants for 10 days. Table III shows the results of the experiment. The dilution end-point apparently lies somewhere between 10^{-3} and 10^{-4}, as compared with 10^{-2} found in the experiments presented in Table I.

TABLE III.

Dilution end-point of potato leaf roll virus in haemolymph of viruliferous Myzus persicae.

Approx. 0.003 μl of diluted haemolymph from viruliferous aphids inoculated into virus-free aphids. Groups of 5 aphids caged on test plants for 10 days.

Dilution	Plants infected/total tested
1 : 10	4/10
1 : 100	2/10
1 : 1.000	1/10
1 : 10.000	0/10

Serial passages. Since the number of aphids that could be injected at one time was quite insufficient for the preparation of suitable amounts of juice for subsequent inoculations, it proved impossible to perform serial passage experiments in exactly the same way as BLACK & BRAKKE (1952) and MARAMOROSCH (1952) did with leafhoppers. The possibility, however, of transmitting potato leaf roll virus by transferring haemolymph from one aphid to another, permitted a new approach.

The experiments were designed as follows. A sample of haemolymph was drawn from a viruliferous aphid and re-injected into 4—6 virus-free insects, each receiving approx. 0.003 μl. Subsequently, the inoculated aphids were caged on Chinese cabbage (*Brassica pekinensis* (Lour.)) plants, which are known to be immune to leaf roll (MACCARTHY, 1954; DAY, 1955b). After one week the surviving insects were removed from the Chinese cabbage and one of them used as donor of a fresh sample of haemolymph to be injected into a second group of virus-free insects. The donor was then tested individually for infectivity by allowing it to feed for 24—72 hours on a healthy young *Physalis* plant, while the second group of inoculated aphids was confined to Chinese cabbage for a week. After this week one of the survivors donated haemolymph for the third injection, was tested for infectivity, and so on. After each series of inoculations the injection needle was either sterilized by immersion into boiling water or replaced by a new one. The experiments were carried on for 19 weeks, during which a total of 1,927 inoculations was performed. Mortality in the injected aphids was 69 per cent as compared with 35 per cent in the controls. Controls of test plant material and virus-free insect stock were performed routinely during the course of the experiments.

A selection from the transmission data obtained is presented in Table IV. The virus could be maintained in the aphids without replenishment from exogenous sources through as many as 15 successive passages. A careful estimate of the dilution which the original virus underwent at each passage gave the value 1 : 25, assuming an average volume of the aphids used of 0.08 μl, and that the virus was not distributed completely uniformly through the insect tissues.

It will be noted from the data of Table IV that the virus was sometimes carried through passages in which the injected aphid did not infect the test plant. Occasionally this might have been due to the fact that the aphid died before it could transmit the virus. An alternative explanation would be variability in efficiency of transmission. That such a variability exists is demonstrated by the results of an experiment in which the aphids, after a 6 hours' feeding period on infected *Physalis*, were kept for various periods on Chinese cabbage. Infectivity of individual insects was tested by caging them for 24 hrs. on test plants. In order to eliminate as far as possible the effect of changing temperatures on virus transmission, the experiments were performed in thermostatically controlled rooms. Two temperatures were used, viz. 30° and 25° C.

The results of these experiments are shown in Fig. 1. Obviously the efficiency of transmission shows large variations. It is tempting to conclude that the frequency at which these variations occurred was higher at 30° than at 25° C., although a definite conclusion has to be postponed until more detailed data are available.

All controls concerning the test plant material and virus-free insect stock, performed regularly in the course of the experiments, remained negative. 26 Chinese cabbage plants, used throughout the whole series of experiments, were examined

TABLE IV.

Serial passage of potato leaf roll virus through Myzus persicae.

Virus-free aphids inoculated with appr. 0.003 μl. of haemolymph from viruliferous aphids. Inoculated insects kept 7 days on Chinese cabbage; haemolymph then collected from one of the survivors and inoculated into another group of virus-free aphids, again caged for 7 days on Chinese cabbage, and so on. After having donated haemolymph the injected insects were tested individually for infectivity on *Physalis floridana* test plants.

— = insect did not infect test plant; + = insect infected test plant;

First symbol of each group refers to insect used as donor. Figures in brackets refer to number of insects injected.

Expt. no.	I	II	III	IV	V	VI	VII	VIII	IX	X
Passage no. 1	— (3)	——— (5)	— (5)	——— (4)	— (3)	—++ (4)	++ (4)	+ (3)	—— (4)	+ (4)
2	++ (3)	— (5)	+ (3)	— (4)	++ (5)	— (4)	++ (4)	— (3)	—— (4)	+ (3)
3	—— (4)	+ (4)	+ (4)	+— (5)	— (5)	+ (4)	+ (4)	————(6)	+ (6)	++ (4)
4	+ (5)	++ (5)	—+ (5)	——— (5)	— (3)	— (4)	+ (4)	— (4)	+ (5)	+ (5)
5	+ (4)	++ (4)	+ (4)	+ (5)	+++ (5)	—+ (4)	+++ (5)	+ (6)	+ (4)	+ (4)
6	++ (4)	+ (5)	+ (3)	— (4)	+— (4)	+ (4)	++ (5)	+ (4)	++ (4)	
7	+ (4)	+ (4)	+ (4)	+ (3)	+ (4)	+ (4)	+ (4)	+ (5)	+ (5)	
8	— (4)	+ (4)	—+ (4)	+ (4)	++ (3)	++ (4)	+ (3)	++ (3)	+ (5)	
9	+ (4)	+ (4)		+ (4)	+ (5)	+ (4)	+ (4)	+ (3)	++ (5)	
10	— (4)	+ (2)		+ (4)	+ (5)	+ (3)	—+ (4)	+ (3)	+— (4)	
11	++ (4)	+ (3)		+ (5)	+ (5)	+ (4)	— (4)	++ (4)	—— (5)	
12	— (3)			+ (5)	++ (4)	+ (4)	++— (5)	+ (4)	—— (5)	
13	— (5)			—+ (4)	—+ (5)	—— (4)	+ (5)	+ (4)	+ (4)	
14	+— (4)			+ (4)	— (4)	— (4)		+ (4)	— (4)	
15				— (3)	+ (3)	++ (4)		+ (4)	++ (5)	

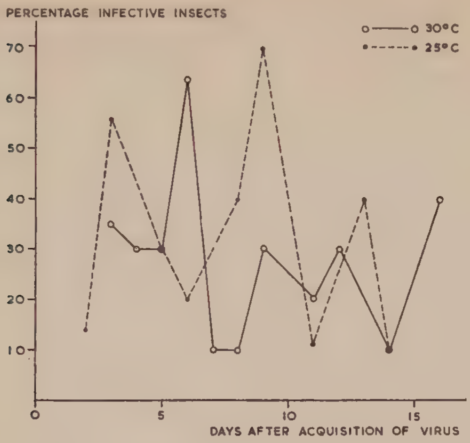

Fig. 1. *Efficiency of transmission of potato leaf roll virus by* Myzus persicae. Large groups of aphids allowed 6 hrs. feeding on diseased *Physalis,* and then caged on Chinese cabbage. Samples of aphids taken at regular intervals and each insect tested individually for infectivity on young *Physalis* test plants. Temperature kept constant at 30° or 25° C. Test plants subsequently kept in the greenhouse.

for the possible presence of virus by colonizing them with groups of 10 virus-free aphids, which subsequently were transferred to healthy test plants. One of these test plants developed leaf roll symptoms, which, however, to all probability was due to contamination.

Discussion

According to Day (1955b) the transmission of potato leaf roll virus by the aphid *Myzus persicae* can be characterized as follows:

I. The aphid retains its ability to transmit the virus following a moult.
II. The aphid's haemolymph contains the virus.
III. The vector is infective for many days, perhaps for its whole lifetime.
IV. There is a well-marked vector specificity.
V. There is often a latent period between acquisition and transmission of the virus by the aphid.
VI. The insect is able to support a limited reproduction of the virus.

Regarding points I, III, and IV the evidence obtained by various authors, e.g. Smith (1929; 1931), Elze (1931), MacCarthy (1954), and Day (1955b) leaves little doubt as to the validity of the statements made. The reported recovery of virus from the haemolymph of infective aphids, was fully confirmed by the present results.

The occurrence of a latent period (V) in potato leaf roll transmission has been the subject of much controversy. The data presented in this paper (Table I) constitute definite proof of the existence of an incubation period following injection of small amounts of virus. Following "normal" acquisition of virus from diseased *Physalis floridana* we found an incubation of approximately 20 hrs., which is in good agreement with the value obtained by Day (1955b).

The length of the incubation period following the injection of virus appeared to depend on the amount of virus injected. MARAMOROSCH (1950) reported a similar dosage effect with aster yellows virus injected into *Macrosteles fascifrons*. The occurrence of this effect is considered as an indication that the virus multiplies within the insect.

The serial passage experiments described in the present paper seem to furnish fairly conclusive evidence of multiplication of potato leaf roll virus in *Myzus persicae* (VI). The dilution end-point of haemolymph from viruliferous aphids was found to be 10^{-4}, whereas the virus could be carried through 15 passages, in which it would have been diluted 10^{-21}. This could have succeeded only in the event of multiplication of the virus in the vector.

The only reservation that can be made — this also holds for part of the work done with Cicadellids — is that the injected insects have had access to plants. Although the immunity of Chinese cabbage seems beyond doubt, it must be admitted that the theoretical possibility of multiplication of the virus in this plant cannot be excluded. Only propagation of the virus in surviving vector tissue in vitro, as demonstrated by MARAMOROSCH (1956b), seems to constitute final proof.

In the preceding section mention was made of the occasional failure of aphids to infect test plants, although virus could be recovered from their haemolymph. Similar observations were made by FUKUSHI (1939) and BLACK (1950). Apart from the explanation furnished by the assumption of the premature death of the insect, it was stated that variations in the efficiency of transmission might be responsible too. Indications for such variations were actually found. FUKUSHI (1939) also held variations in the insect's ability to transmit the virus responsible for the observed anomalies. His opinion was that as a result of occasional low temperatures virus multiplication in the insect was delayed, so that the concentration of virus in the salivary glands could drop below the critical level. Fig. 1 shows that variations in transmitting power are also encountered at constant temperatures. Perhaps this indicates a certain discontinuity in the process of virus multiplication, not unlike the "bursts" in bacteriophage reproduction.

Recently, HARRISON (1958) reported the failure to detect any multiplication of potato leaf roll virus in *Myzus persicae*. On the contrary, the concentration of virus in the insect apparently decreased during its stay on an immune host. It seems reasonable to suppose that, when virus multiplication in the insect proceeds at a limited rate (which seems to be true in the present case), the demonstration of an actual increase in virus concentration will depend greatly on the rate at which the virus is egested by the insect. The demonstration of a net decrease in virus concentration *per se* cannot be considered as a conclusive argument against multiplication of potato leaf roll virus in *Myzus persicae*.

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ZUSAMMENFASSUNG

1. Das Blattrollvirus der Kartoffel konnte durch Injektion von Preßsaft oder Blut virusinfizierter *Myzus persicae* (Sulz.) auf virusfreie Insekten übertragen werden.
2. Bei Serien-Übertragungen unter Verwendung von Blutinjektionen blieb das Virus in 15 aufeinander folgenden Generationen ursprünglich virusfreier Blattläuse erhalten. Hierbei wurde nach der Injektion als Zwischenwirt der blattrollunempfindliche chinesische Kohl benutzt.
3. Die theoretische Verdünnung der ursprünglichen Virusmenge pro Blattlaus betrug nach der 15. Passage ungefähr 10^{-21}. Injektion des auf $1:10^4$ verdünnten Blutes virusinfizierter Läuse ergab dagegen in keinem Fall Infektionsfähigkeit (Tabelle III und IV).
4. Die Existenz einer Latenz-Periode bei der Übertragung konnte nach Injektion kleiner Virusmengen bewiesen werden. Verdünnung des injizierten Blutes verlängerte diese Periode (Tabelle I und II).
5. Diese Befunde deuten auf Vermehrung des Virus innerhalb der Blattläsen.
6. Wenn virusinfizierte Blattläuse auf chinesischem Kohl gehalten werden, zeigt ihre Infektionsfähigkeit deutlich periodische Schwankungen (Fig. 1).

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OBSERVATIONS ON THRESHOLD INTENSITY AND SENSITIVITY TO DIFFERENT WAVE LENGTHS OF PHOTOPERIODIC RESPONSES IN THE COLORADO BEETLE *(LEPTINOTARSA DECEMLINEATA* SAY)

BY

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The threshold intensity of white light for photoperiodic responses of the Colorado beetle is below 0,1 lux (0,4 ergs/cm^2/sec.). Above this value, the responses increase with the intensity until above 5 lux (20 ergs/cm^2/sec.) they remain more or less constant. Experiments with filtered light (423—675 mμ) and infrared ($>$ 700 mμ) revealed that even 675 mμ is clearly active.

With the growing interest in photoperiodic responses of insects there is an increasing demand for experimental data on the sensitivity of these responses to light of narrow spectral regions and various intensities.

Knowledge of these relations may contribute to our understanding of the ecological significance of the photoperiod. It also stands at the base of any physiological analysis of the photodynamic processes involved.

It seems superfluous to review the literature pertaining to our subject, as this has recently been done by LEES (1955). After his book was issued, experiments have been published by GEYSPITZ (1957) on sensitivity to light of different wavelengths of photoperiodic responses in *Pieris brassicae* L., *Dendrolimus pini* L., and *Acronycta rumicis* L. Using absorption filters, GEYSPITZ could show that *A. rumicis* reacts to wavelengths ranging from 4.070—6.550 A.U., while in *P. brassicae* and *D. pini* 6.550 A.U. is practically equal to darkness.

The insensitivity to red, infrared and ultraviolet and the maximum observed in the blue and blue-green regions of the spectrum observed with several species have led LEES (1955) to the supposition that carotenoïds may act as sensitizing pigments.

The general impression up till now is, that the threshold of perception for white light is usually ± 10 lux, though this value has been determined under very variable conditions. Thus, in *Grapholita molesta* an intensity of 10—30 lux at a minimum was required when radiated upon the surface of apples in which the larvae were tunneling. Apparently, the larva itself has a much lower threshold (DICKSON, 1949).

The silkworm egg has a threshold of only 0,1 lux, the larva of 0,8 lux (KOGURE, 1933).

In the red spider mite, *Metatetranychus ulmi* (Koch), the threshold is between 10 and 20 lux (LEES, 1953).

This publication must be considered as a progress report of a study which is being continued. We have started from the observation that in the adult Colorado Beetle, diapause is induced by photoperiods of 15 hrs. and less, while photoperiods of 16 hrs. and above lead to an increasing percentage of active, reproducing beetles (DE WILDE, 1954, 1958).

EXPERIMENTAL

The action of different wavelengths and intensities was studied by means of the system of supplementary photoperiods. Under this *régime* a short-day effect is obtained when the experimental illumination is inactive, while in the opposite case, a long-day effect is produced.

In all experiments, the insects were subjected *ab ovo* to the following schedule of illumination:

9 a.m. — 5 p.m. normal daylight
5 p.m. — 12 p.m. experimental light
12 p.m. — 4 a.m. darkness
4 a.m. — 9 a.m. experimental light.

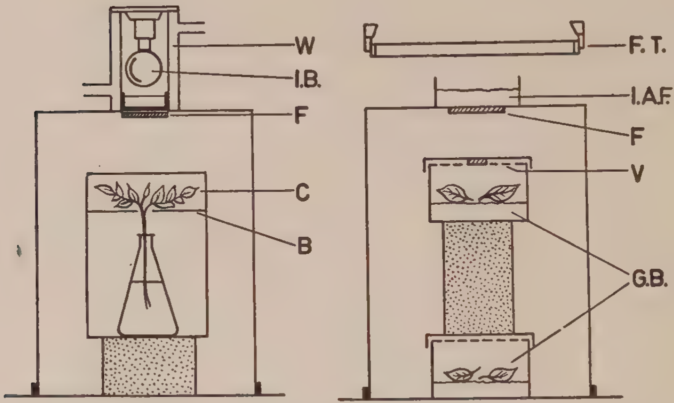

Fig. 1. Scheme of the experimental set-up for studies with filtered light. W = Water mantle; I.B. = Incandescent bulb; F = Interference filter; C = Beetle compartment; B = Cardboard screen; F.T. = fluorescent tube; I.A.F. = Infrared absorption filter; V = Ventilation system; G.B. = Culture dish at different intensities for rearing larvae.

This schedule is represented in fig. 3 (p. 304). During the daylight-period, food was changed and further handling of the cultures was performed.

Groups of 100 larvae were reared *ab ovo* in open crystallizing dishes placed under light-proof, tin or cardboard cylinders (figs. 1 and 2).

Water cooled, 25 W. incandescent Philips bulbs (Exp. I) or fluorescent 40 W. Philips lamps (Exp. II) placed on top of the cylinders were used as light sources.

Various intensities of white light were obtained by alternating layers of glass

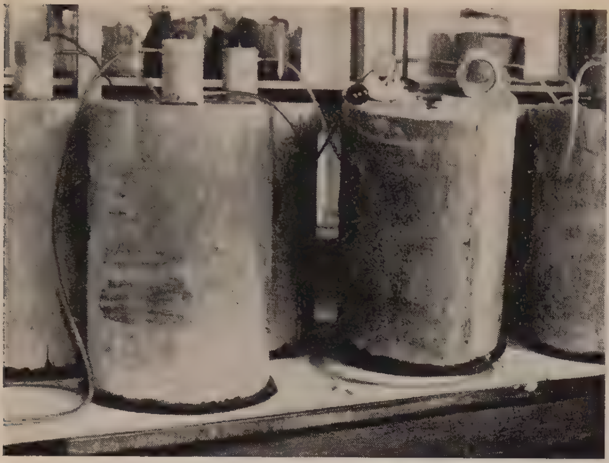

Fig. 2. Metal cylinders with cooled illumination system as used in our experiments.

and white paper. In the experiments with filtered light, use was made of Schott interference filters, details of which are given in Table I.

TABLE I

Properties of the Schott interference filters used in our experiments

no.	colour	range of transmission (mμ)	maximum transmission (mμ).
608.707	violet	400—430	423
345.101	ultramarin	430—460	458
619.332	eisblau	460—495	480
383.810	blaugrün	495—520	504
384.103	grün	520—550	538
385.312	gelbgrün	550—575	560
017.758	gelb	575—595	585
608.212	orange	595—625	606
624.825	hellrot	625—660	646
610.777	dunkelrot	660—700	675

When the characteristic of the filters was checked, it was found that, if use is made of incandescent bulbs, the filters transmit a considerable quantity of infrared radiation in addition to the spectral region mentioned by the producers. Therefore, additional experiments were made with infra-red light. This was obtained by the use of a compound filter made of red and blue glass, a characteristic of which is given by STOLWIJK (1954). It exclusively transmits above 700 mμ.

A Lange photo-electric lux meter was calibrated to each of the filters [1]). As the lux is not a unit of energy, it was preferred to express the illumination in ergs/cm^2/sec. For this purpose, the number of ergs/cm^2/sec. corresponding with one scale unit of the lux meter was determined for each of the different spectral regions. For white light within the region of 400—700 mμ, one lux approximately corresponds with 4 ergs/cm^2/sec.

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[1]) The help and advice of Prof. Dr. E. C. WASSINK and Ir. P. GAASTRA in choice of filters and light measurements is gratefully acknowledged.

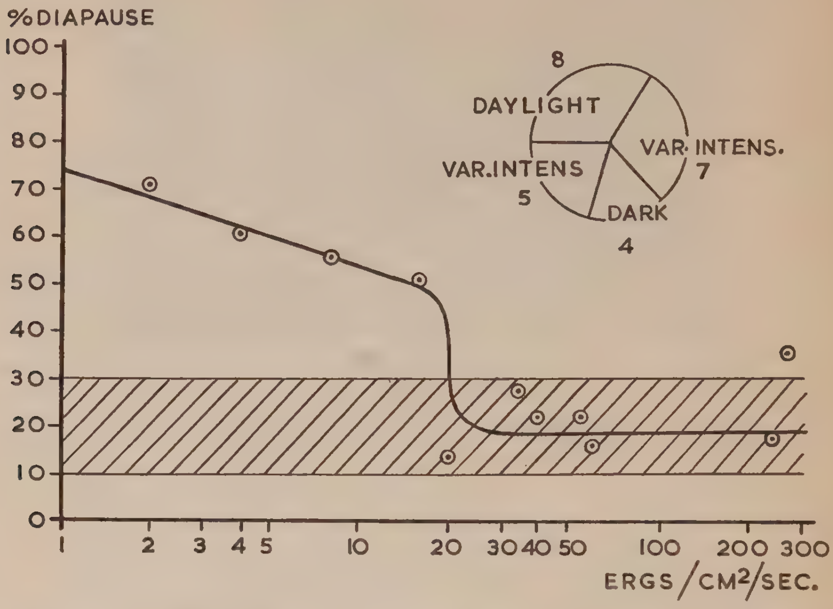

Fig. 3. Effect of different intensities of white light as used in our illumination program. The shadowed region corresponds with the normal variation of the diapause % under "long-day" treatment with high intensities.

The intensities given in Table I are measured at the level of the experimental animals placed upon fresh potato leaves as a food. This situation is shown in fig. 1. It follows that the values actually received are often less as the beetles sometimes hide between the leaves.

RESULTS

a. Threshold intensity for white light

The results of three series of experiments are summarized in Table II and shown graphically in fig. 3. As described elsewhere (DE WILDE, 1954, 1958) former experiments have shown that under "long day" conditions up to ± 30% of the beetles may still enter diapause. It follows that the threshold is below 0,1 lux (0,4 ergs/cm^2/sec.). Above this value, the effect increases with the intensity until at 5 lux (20 ergs/cm^2/sec.) it remains more or less constant.

b. Sensitivity to light of different wavelengths

As mentioned before, all experiments were made with an intensity of 40 ergs/cm^2/sec. As this is not far above the critical value of 20 ergs/cm^2/sec., it might be expected that the diapause percentage and egg-production observed

TABLE II

Results of the experiments with white light of varying intensity (Program of illumination, see fig. 3)

		No. of active beetles				No. of diapause beetles				
		Exp. no.				Exp. no.				
ergs/cm²/sec.	lux	1	1a	2	tot.	1	1a	2	tot.	% diapause
0	0		1	3	4		22	29	51	93
0,4	0,1			7	7			24	24	77
0,8	0,2	5	8		13	11	28		39	75
2	0,5			8	8			20	20	71
4	1	2	10	3	15	2	19	3	24	61
8	2	3	13		16	2	18		20	56
16	4	11	13		24	11	14		25	51
20	5			26	26			4	4	13
34	8,5	12	26		38	6	9		15	28
40	10			22	22			7	7	24
56	14	16	22		38	4	8		12	24
60	15			29	29			5	5	15
240	60			26	26			5	5	16
264	66	13			13	7			7	35

with different wavelengths would reflect the sensitivity to different wavelengths at least superficially.

The results are given in Table III and fig. 4.

TABLE III

Results of experiments with filtered light. Program of illumination see text. Intensity 40 ergs/cm²/sec. Duration of experiments 27 days.

Max. transmission (mμ)	Active			In diapause			% diapause	Eggs p. ♀
	Exp. 1	Exp. 2	tot.	Exp. 1	Exp. 2	tot.		
423	14		14	7		7	33	301
458	12	3	15	9	1	10	40	269
480	25		25	1		1	4	142
504	21		21	2		2	9	260
538	17	10	27	2	3	5	16	182
560	16	6	22	3	2	5	19	198
585	20	30	50	6	2	8	14	165
606	21	30	51	3	5	8	14	115
646	16	12	28	1	2	3	10	339
675	15	23	38	8	9	17	31	102
>700	4		4	27		27	87	39

Two points may be mentioned:

1. An activity is shown in all regions of the visible spectrum ranging from 423—675 mμ, while the effect of infrared is negligeable.
2. The effect is constant from 480—646 mμ and definitely diminishes above 646 mμ.

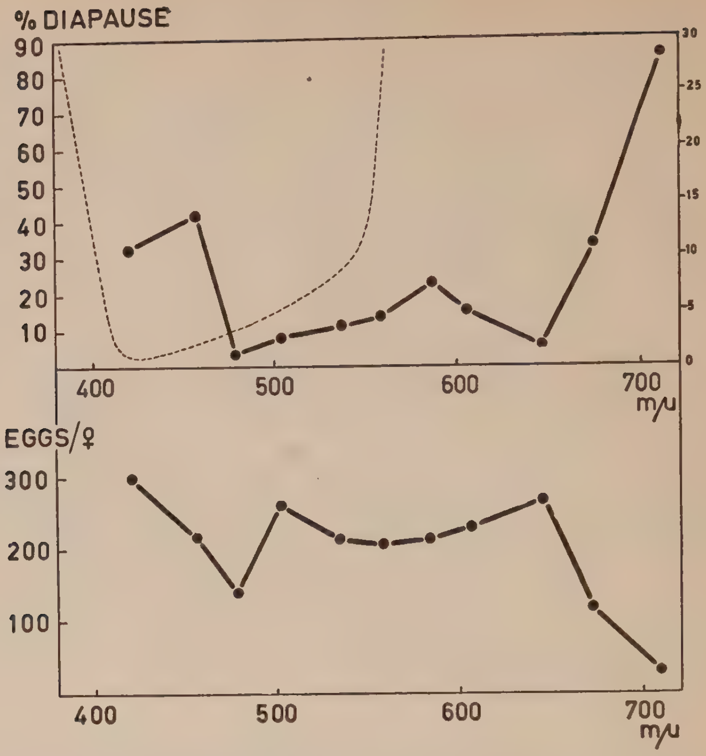

Fig. 4. Effect of filtered light on diapause and oviposition as found in Exp. I. Illumination programme see fig. 3. The broken line shows the relative energy required with different regions of the visible spectrum to obtain a photoperiodic effect in *Metatetranychus ulmi* (LEES, 1955).

CONCLUSIONS

In a range between 0,1 and 5 lux (0,4—20 ergs/cm^2/sec.) the effect of white light on the photoperiodicity of the Colorado beetle increases with its intensity. Above this value no further increase takes place.

As the intensity of direct moonlight usually falls within the range of 0,1—0,5 lux (LEES, 1955) the question presents itself whether, at least during full moon, this light may act as a supplement to the daily photoperiod.

As regards sensitivity to filtered light, we have not yet been able to determine the relative energy required for different wavelengths to obtain a constant effect. Nevertheless, it appears that, compared with *Metatetranychus*, the active waveband for *Leptinotarsa*, extends much further into the red.

ZUSAMMENFASSUNG

1. Die Intensitätsschwelle für weisses Licht in Bezug auf seine photoperiodische Wirksamheit auf den Kartoffelkäfer (*Leptinotarsa decemlineata* Say) liegt unterhalb 0,1 Lux (0,4 ergs/cm^2/Sek.).
2. Bis zu 5 Lux (20 ergs/cm^2/Sek.) wirkt sich erhöhte Lichtintensität in steigerndem Masse aus; oberhalb dieses Wertes wird der Diapauseprozentsatz mehr oder weniger konstant.

3. Versuche mit Spektrallicht von 423—675 mμ und Infrarot (>700 mμ) konstanter Intensität (40 ergs/cm²/Sek.) ergaben, dass sogar 675 mμ noch deutlich wirksam ist. Im Bereich von 423—646 mμ war das Spektrallicht ohne wesentlichen Unterschied wirksam.
4. Es sind Experimente geplant, die ein relatives Energiespektrum für photoperiodische Wirkung verschiedener Wellenlängen ergeben sollen.

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THE ECOLOGY OF SYMPHYLA

PART I. POPULATIONS

BY

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Symphylid populations were studied using a standardized sampling technique, consisting of twenty random, vertical 2½″ diameter cores taken to the subsoil, and flotation extraction. Of 415 locations sampled symphylids were present in 32.0% of grassland, 25.8% of forest litter, 44.2% of cultivated soils and 52.8% of greenhouse soils. Populations were high, up to 88,000,000 per acre, and the order of decreasing population density was greenhouse soil, outdoor cultivated soil, forest litter and fallow soil. Investigations on optimal soil type showed loams to be most favourable, pure sands and clays least suited, and clay loams and sandy clay loams intermediate. It was concluded that the most favourable soil characteristics for symphylids were high organic matter content, good moisture holding capacity and the ability to compact and form crevices.

Symphylids are often regarded as rare animals, but this is more a reflection of their small size and the lack of information about soil animals in general, than a true picture of their abundance in nature. Other small soil animals, such as the Protura and Pauropoda are also relatively unfamiliar, and were seldom recorded in the earlier studies of the soil fauna. STARLING (1944) however, showed that the Pauropoda could be very abundant in forest litter, and RAW (1957) found large populations of Protura in grassland. In the scope of this paper it is demonstrated that symphylids are common in a wide range of soil habitats.

In early studies of the entire soil fauna, samples of soil were sorted by hand in order to obtain estimates of the population density and distribution. It is only in comparatively recent years that more efficient soil sampling techniques, and different methods of extracting arthropods from soil, have given some idea of the immense numbers of invertebrates present in many soils. Extraction methods are far from perfect even now, and a technique which satisfactorily extracts some types of arthropods from a particular soil may be totally unsuitable for others. It follows therefore that estimates of the total numbers of invertebrates present in soils, obtained by use of a single extraction method are probably extremely conservative.

Early workers on the soil fauna such as CAMERON (1931), did not report Symphyla at all. Later investigators such as MORRIS (1922, 1927) and E. E. EDWARDS (1929) estimated only small numbers of symphylids to be present in the soils they studied. Many of these early workers based their estimates on a few large soil samples and separated the animals from the soil by hand. Small organisms such as symphylids are easily overlooked in such work.

Records of symphylids from the literature on general soil faunal studies, are summarized in Table I. Where the authors have not given an estimate of the population density of symphylids, calculations have been made from their data

TABLE I

Proportions of Symphylids present in total arthropod populations

Author	Habitat	Method of extraction	No. of invertebrates (millions/acre)	*Myriapoda* percentage of total invertebrates	*Symphyla*	
					percentage of total invertebrates	percentage of *Myriapoda*
MORRIS	1922 Unmanured pasture	Hand sorted	3.62	24.2	1.8	7.3
	Manured pasture	Hand sorted	10.94	16.4	2.0	11.3
THOMPSON	1924 Pasture	Wet sieved and flotation	19.5	small	small	86.0
MORRIS	1927 Unmanured pasture	Wet sieved	1.21	7.9	0.3	3.1
	Manured pasture	Wet sieved	5.10	36.2	0.7	2.0
E. E. EDWARDS	1929 Arable and Grassland	Wet sieved and flotation	27.2—44.6	small	small	large (most in arable)
SAWA	1930 Arable	Hand sorted and flotation	—	—	0.8	79.7
	Grassland	Hand sorted and flotation	—	—	0.2	43.9
VAN ZWALUWENBERG	1930 Sugar cane	Hand sorted	70.0	25.2	5.6	—
	Fallow	Hand sorted	—	26.9	9.3	—
BAWEJA	1939 Arable	Flotation	67.6—111.8	18.0	0.06	0.3
WILLIAMS	1941 Forest litter	Flotation (Ladell)	15.5—40.0	3.13—3.52	0.21—0.26	5.9—8.3
STARLING	1944 Forest litter	Tullgren funnel	—	—	—	16.6
PEARSE	1946 Forest litter	Tullgren funnel	—	—	—	37.1
SALT et al	1948 Grassland	Sieved and flotation	1,068.8	2.1	1.5	69.5
SALT	1952 Grassland	Sieved and flotation	—	—	—	67.0
	Coffee plantation	Sieved and flotation	—	—	—	63.0

where possible, both of the size of the symphylid population and of the total arthropod population. Considerable variation is shown by these figures, but in the more exact studies of THOMPSON (1924), PEARSE (1946), SALT, HOLLICK, RAW & BRIAN (1948) and SALT (1952), symphylids comprised between 37% and 86% of the total myriapod population. In most studies they represented less than 2% of the total arthropod population. On this basis it would appear that symphylids while being quite common, do not attain very high population densities.

A number of factors, however, may be responsible for the low numbers of symphylids estimated by these workers. None of the methods used were very satisfactory for extracting symphylids from the soil. Many symphylids may be overlooked by hand sorting due to their small size, while in wet sieving these fragile animals are easily damaged, and from most soils, Tullgren funnels appear to extract only a very small percentage of the total population. Secondly, in none of the investigations were samples taken to a depth of more than twelve inches below the soil surface. Most workers were under the impression that soil animals were rare below this depth, e.g., THOMPSON (1924) states "Under normal or very moist conditions, organisms were rare below nine inches deep, and a few Symphyla and Achorutids occurred at a depth from nine to twelve inches but not lower". Later population studies have indicated that symphylids are actually more common in the lower soil strata than in the upper, SALT *et al* (1948) and MICHELBACHER (1939), and they frequently descend to a depth of several feet. Furthermore symphylids have very definite vertical migrations in the soil in response to environmental changes, WYMORE (1924). Finally, symphylid aggregations are common and a few large samples could well miss these and lead to the conclusion that these animals were totally absent, or only present in small numbers.

The work of MICHELBACHER (1938, 1939, 1949) gave a much clearer picture of the size of symphylid populations which might occur, and of their vertical distribution in the soil. His data, together with population estimates obtained from the work of other authors, are given in Table II. It can be seen from this table that, in general, flotation extraction methods of symphylids from soil gave much larger population estimates than those obtained by other methods. This was confirmed by C. A. EDWARDS (1955) who compared the numbers of symphylids extracted from corresponding soil samples by flotation and by Tullgren funnel. For all the soils examined, the flotation method gave a much higher percentage extraction.

All these previous investigations have been carried out on a few isolated sites and without reference to soil type, cultivation or other soil variations. In the present studies samples have been taken from a large number of sites, and from these, estimates of the size of symphylid populations have been made. Since some species of symphylids are economic pests and others not, it was expected that differences would show up in their habitat preferences. Consequently population estimates have been related to soil type and to the type of cultivation practised. In a later paper it is proposed to outline the relative importance of the environmental factors responsible for the complicated vertical migrations of symphylids

TABLE II

Summary of Symphylid populations from the literature

Author	Date	Habitat	Depth of sample	Method of Extraction	Population of symphylids	
					Millions/acre	No./sq. meter
Morris (in Russell)	1923	Unmanured pasture	9″	Hand sorted	0.064	16
		Manured pasture	9″	Hand sorted	0.215	53
Morris	1927	Unmanured pasture	9″	Hand sorted	0.003	0.8
		Manured pasture	9″	Hand sorted	0.036	9
Sawa	1930	Arable	11″	Hand sorted and flotation	1.268	313
		Pasture	11″	Hand sorted and flotation	0.171	42
Van Zwaluwenberg	1931	In sugar cane	Surface	Hand sorted	3.920	968
		Fallow soil	Surface	Hand sorted	6.540	1,604
Baweja	1939	Arable (Allotments)	9″	Ladell flotation	0.042	10
Michelbacher	1939	Cultivated	18″	Flotation	2.705	668
Symphylella sp.		Field crops	18″	Flotation	4.915	1,212
		Field crops	18″	Flotation	22.700	5,440
		Field crops	18″	Flotation	8.000	1,976
		Glasshouse soil	18″	Flotation	90.300	22,200
Salt et al	1948	Pasture	12″	Wet sieved and flotation	15.675	3,870
Pearse	1946	Forest litter	9″	Tullgren funnel	0.414	99
C. A. Edwards	1955	Cultivated soil	To subsoil	Flotation	4.220—20.480	1,042— 5,065
		Glasshouse soil	To subsoil	Flotation	2.560—76.800	635—19,000
Belfield	1956	Pasture	18″	Ladell flotation	3.25—9.08	804— 2,240

in the soil, but at present this has been omitted, and accounted for by taking very deep samples which should include all symphylids present.

SAMPLING TECHNIQUE

Symphylids have a non-random distribution in soil. Coefficients of dispersion calculated by SALT *et al* (1948) were significantly greater than unity. In the present work calculations of χ^2 from the sampling data obtained from a number of different sites, indicated a highly significant departure from a random distribution. The aggregations appear to be due to a variety of factors such as soil structure, organic status, temperature and the presence of breeding groups, but more particularly to the presence of abundant moisture in the soil, as will be shown in a later paper. In view of this emphasis was placed on a relatively large number of smaller samples, and deep samples were taken whenever possible, right down to the subsoil. Comparison of data from a number of sets of sampling on the same site showed that twenty random, vertical, two and one half inch diameter cores, taken over an area of five hundred square yards, gave estimates for the population that did not usually differ significantly. The samples were taken with a sampling tool consisting of a metal cylinder 2½″ in diameter and 2½″ deep, slightly narrowed towards the cutting edge to facilitate the removal of soil. This was attached to a metal handle three feet long. With this tool it was possible to take successive vertical cores using marks on the handle of the tool 3″ apart as guides, and by keeping these samples separate, the vertical distribution of symphylids in any sample could be determined.

It was found to be important in taking the samples that they were completely at random, and that they were taken at a time when the soil surface was either uniformly covered with vegetation or grass, or when no vegetation at all was present. If this was found to be impossible then half the samples were taken in the open soil and half among the roots of the plants. In this way the primary root feeders, which are attracted to the plant roots, and the saprophagous species feeding on decaying material around the roots, would be accounted for, and a fair estimate of the total population made.

EXTRACTION TECHNIQUE

Symphylids readily float on water and this greatly simplifies their extraction from many types of soil. In much of the work it was possible to drop the sample directly into a container of water, stir gently, and count the symphylids as they floated to the surface. Alternatively, the samples were placed in polythene bags until they could be dealt with in the laboratory. In soils which gave rise to an excessive amount of froth on the water surface, the floating matter was run off into a 150 mesh sieve, washed under a stream of water to disperse the froth and then refloated on clean water when the symphylids could be easily picked off with a brush. Where very accurate estimates of the population were required a modified Ladell apparatus was used, C. A. EDWARDS & DENNIS (1958).

EFFECT OF CULTIVATION ON POPULATIONS

A number of workers on the Symphyla have expressed opinions on the type of cultivation which favours the build-up of large populations, but none of them have given numerical data to support these ideas. Furthermore many statements have been extremely conflicting. NEWPORT (1845) thought that symphylids were commonest in light leaf mould at the roots of grass, and GRASSI (1886), BAGNALL (1913), and FRIEDEL (1928) believed that they were most common under stones, the former author expressing the opinion that they were never found where there were no stones. WILLIAMS (1907), and MICHELBACHER (1949) thought that uncultivated or undisturbed soils offered a more favourable environment for the development of most species of symphylids than cultivated soils. THOMPSON (1924) also thought that symphylids were less common in cultivated land than in pasture, but E. E. EDWARDS (1929) believed that cultivation increased both the myriapod and symphylid populations. This latter view was supported by SAWA (1930) who found many more symphylids in arable land than in grassland, although his observations were confined to two fields. DAVIS (1924) and MORRIS (1922) recorded higher populations of symphylids where manure had been added to the soil than in control plots. Many workers have observed that symphylids are common in forest litter and rotting logs, MENGE (1851), EATON & CHANDLER (1942), PEARSE (1946), STARLING (1944) and WILLIAMS (1941). FRIEDEL (1928) thought that larger populations occurred in beechwoods, pinewoods and spruce woods, than in pastures and meadows. VAN ZWALUWENBERG (1931) found greater populations in fallow soil than in a field growing sugar cane but MICHELBACHER (1938) found higher numbers of *Scutigerella immaculata* (Newport) among cultivated plants than in fallow soil at a number of sites.

TABLE III

Occurrence of symphylids in relation to habitat

Habitat	No. of sites with *Scutigerella* spp. present	No. of sites with *Symphylella* spp. present	No. of sites with both spp. present	No. of sites with no symphylids present	Percentage of habitats with symphylids
Grassland	3	5	0	17	32%
Forest litter	5	2	1	23	26%
Cultivated soil	27	47	9	103	44%
Greenhouse soil	24	49	18	81	53%
Total (% of all habitats)	14%	25%	7%	54%	

In the present work 415 sites were sampled for symphylids and Table III summarises the types of cultivation and the occurrence of symphylids under these conditions. Symphylids were found in 46% of all the sites sampled, *Symphylella* spp. being commoner than *Scutigerella* spp. and in 7% of sites, specimens of both genera were found together. Several other genera of symphylids were found in the course of this work, C. A. EDWARDS (1959), but have been omitted from this table since they were relatively scarce. There is probably some bias in the data, in favour of finding symphylids, since much of the sampling was carried out as part of advisory work related to the economic importance of these animals, and samples were often taken where it was suspected that symphylids might be present.

These figures appear to be good evidence that symphylids are extremely common soil animals at least in Southern England. Sampling has also been carried out in the Channel Islands and in Wisconsin, U.S.A., and data obtained from these sites indicate that symphylids are as common there as in S. England. Yet at the present time most counties in England, and many States in the U.S.A., have no faunal records of symphylids.

TABLE IV

Populations of Scutigerella *spp. found in different soil habitats in southern England*

Habitat	No. of sites	Population (millions per acre)		
		Mean number for habitat	Standard Deviation	Range
Fallow soil	3	1.3	0.8	0.5— 1.7
Fallow soil with high humus level	3	3.9	1.6	2.1— 4.8
Grassland	5	4.8	2.3	1.8— 7.2
Forest litter	3	5.8	0.8	4.7— 6.9
Cultivated soil	7	12.1	10.0	4.2—28.5
Greenhouse soil	7	31.4	35.5	5.8—88.2

TABLE V

Populations of Symphylella *spp. found in different soil habitats in southern England*

Habitat	No. of sites	Population (millions per acre)		
		Mean number for habitat	Standard Deviation	Range
Fallow soil	3	4.5	0.7	3.8— 4.6
Grassland	3	1.8	1.3	0.4— 3.0
Forest litter	3	7.4	4.2	5.6—12.2
Cultivated soil	6	10.2	4.6	4.8—16.4
Greenhouse soil	6	31.8	26.2	5.1—73.0

TABLE VI

Populations of Symphylids in different soil types

Habitat	No. of sites	Population (millions per acre)		
		Mean number for soil type	Standard Deviation	Range
Clay	3	0.5	0.7	0.1— 1.3
Clay Loam	6	10.8	7.6	1.4—21.3
Sandy Clay Loam	7	14.6	6.2	4.2—19.7
Loam	10	27.2	32.0	4.6—88.2
Sandy Loam	5	7.3	3.1	3.3—10.8
Sand	2	1.2	0.7	0.7— 1.7

At many locations very thorough sampling enabled population estimates to be made. These are summarized for two genera of symphylids in a range of habitats in Tables IV & V and Fig. 1. The data were analysed after logarithmic transformation and showed significant differences (1% level) in mean population in relation to soil habitat for both genera. In some of the habitats extremely high populations of symphylids were recorded, to the extent that in these locations symphylids may represent a considerable proportion of the total population of soil arthropods.

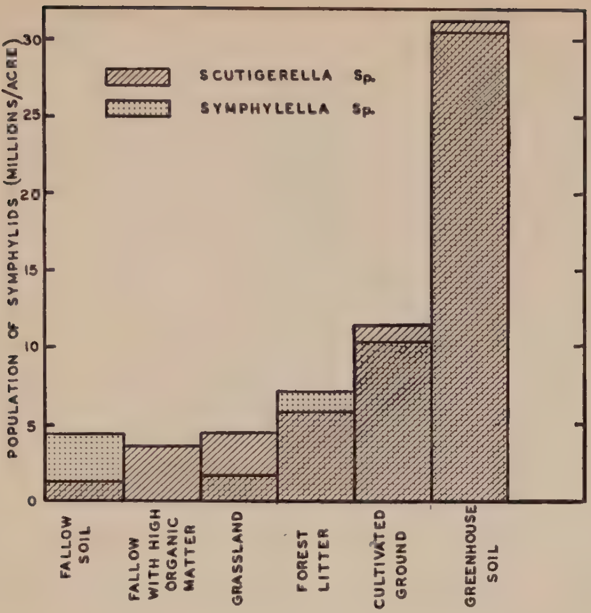

Fig. 1. Populations of symphylids in relation to soil habitat.

EFFECT OF SOIL TYPE ON POPULATIONS

There are no exact data in the literature on optimum soil types for high populations of symphylids. HANSEN (1903), and BAGNALL (1913) expressed the opinion that symphylids were most common on limestone formations in clay soils. MICHELBACHER (1938) was also of the opinion that heavy textured soils were more favourable to symphylids, since they allowed permanent channels to form as a result of decaying rootlets, and worm and insect burrows. The abundance of symphylids in badly drained soils was noted by ILLINGWORTH (1929), and MARTIN (1948) observed that symphylids could move easily through the cracks in clay, whereas sand presented an impenetrable barrier. STRICKLAND (1945) found symphylids in all the soils that he studied in Trinidad, with the exception of light sand and heavy clay. Five different soil types studied by E. E. EDWARDS (1929), in order of increasing symphylid populations were alluvial soil, boulder clay, sedentary soil, lighter drift and arable soils. KEARNS & WALTON (1932) and WALTON (1932) believed that a porous stony soil was the most suitable environment.

In the present studies six common soil types were selected for study, from data available on population densities of symphylids occurring in different soils. The average numbers per acre calculated from sampling data are shown in Fig. 2 and in Table VI. Soils used for cultivated crops favoured symphylid multiplication, and such soils ussually contain a relatively high proportion of organic matter which

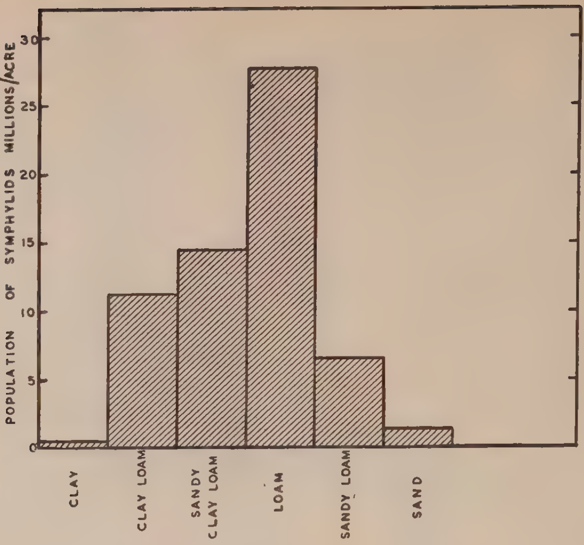

Fig. 2. Populations of symphylids in relation to soil type.

may provide food for the symphylids. Clay loams hold more moisture than sandy soils, and moisture is an important factor in influencing distribution of symphylids. It is also possible that sandy soils have an abrasive action on the delicate cuticle of the symphylids. In the course of sampling it was noted that a very heavy clay subsoil formed an effective barrier to downward migration of symphylids, and in laboratory experiments they were unable to pass through a layer of pure sand.

Discussion

One of the most interesting aspects of these population studies is the stability of the population density on any particular site. Sampling carried out in successive years, generally showed only gradual changes in population numbers if deep samples were taken. Furthermore, lateral movement was only very gradual and limited, the same areas of high infestation persisting over several samplings. In the same way the numerical changes through any particular year were relatively small, the greatest number of symphylids being found in spring. The vertical distribution of a population, however, changed very considerably with the season, for example in November and December a large proportion of the total population of *Scutigerella immaculata* may be in the lower soil strata while in spring it is mainly concentrated close to the surface.

Such stability of population numbers indicates that it is principally the general physical factor of the site which favours the population build up. Superimposed on the suitability of the site, in the case of *Scutigerella* spp., may be a gradual build up in numbers due to a particular cropping rotation, e.g., tomatoes followed by lettuce is a favourable rotation for increase in numbers. A second factor contributing to stable populations is the relatively long life cycle of symphylids. They require four to six months to reach sexual maturity from the egg, and individual symphylids have been kept alive in the laboratory for as long as four years. Breeding occurs throughout the year but reaches its peak in spring.

Although, in general, populations appear to be very stable, an exception occurs where a very high population may completely disappear in the course of several months. Such a decrease may be explained by the onset of disease or a sudden build up of parasites or predators, but this is pure speculation and requires further investigation. Sudden reduction of the population in this way occurred at three of the sites studied. A gradual decrease in population also occurs as the result of routine use of soil insecticides in the case of species of symphylids which are economic pests, but only in very few of the sites where such treatments were carried out was the population completely eradicated. The treatments only affected the surface population, and symphylids in the lower strata kept the whole population from disappearing.

Certain habitats were shown to favour build up of symphylids, and of these greenhouse soil seemed to favour their increase more than other types of habitat, while in general cultivation is favourable to numerical increase. In fact all situations where there was a high percentage of organic matter present tended to favour high population densities.

Of the different soil types, cultivated loams appeared to favour high population densities. Soils of a heavier nature tended to encourage numerical increase, while soils containing a high proportion of sand did not. Very heavy clays and sandy soils were not at all favourable for increase in numbers of symphylids. Some suggestions to explain this distribution can be put forward. Soils used for cultivated crops favoured symphylid multiplication as we have seen and such soils are commonly loams, and contain a high proportion of organic matter. Clay loams hold more moisture than sandy soils, and moisture has a considerable influence in the distribution of symphylids. It is also possible that sandy soils have an abrasive action on the delicate cuticle of symphylids.

In work of this kind, it is very difficult to separate the influence of the different factors on the population as a whole. For instance the highest populations of symphylids occurred in open loams, but these are also the commonest types of cultivated soils, and cultivation also appeared to favour high populations.

It is possible, however, to make some estimate of the relative effects of such factors by observing changes in the population density where only one factor varied at a time, as in the case where a homogeneous piece of land had been only partially cropped. In such cases there was usually a greater build-up of numbers in the cultivated section of land but, contrary to expectations, the build-up was comparable both for the phytophagous *Scutigerella* spp. and the saprophagous *Symphylella* spp. This indicated that some factor resulting from cultivation and not necessarily the abundance of food was influencing the size of the population. Such a factor may well be the provision of an open soil structure providing ready passage for symphylid movement. Further observations of this nature indicated that a high percentage of soil organic matter favoured high populations. It was not possible to study the effect of soil acidity in this way, but during the work it was noted that high symphylid population densities did not occur in very acid soils.

In general, sites which had a very deep layer of soil were more favourable to symphylid multiplication than those with the subsoil close to the surface. On a number of sites, however, large populations did occur in shallow soils and, in one case, where the water table was only a foot below the soil surface. The highest population densities of all occurred in glasshouse soils, and this is probably a feature of the high average soil temperature and relatively constant soil moisture conditions.

It is possible to summarize the conclusions drawn from the observations made in this work as to the optimum soil conditions for large symphylid populations. These would be a well-aerated, neutral, cultivated loam soil with a high proportion of organic matter. The soil should be have the ability to maintain a high average temperature, retain moisture well, and compact sufficiently to permit cracks to appear so as to provide suitable runways to the lower soil strata.

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ZUSAMMENFASSUNG

Unter Anwendung einer standardisierten Sammeltechnik wurden Symphylidenpopulationen untersucht, wobei dem Untergrund jeweils zufallsmäßig 20 vertikale Bohrkerne entnommen und durch Schlämmen ausgezogen wurden. Die an 415 Lokalitäten gesammelten Symphyliden waren zu 32,0% in Grasland, zu 25,8% in Waldstreu, zu 44,2% in Kulturböden und zu 52,8% in Gewächshausböden vertreten. Die Populationen waren groß, bis zu 88 Millionen je Morgen (acre), und die Populationsdichte nahm in folgender Reihe ab: Gewächshausböden, Freilandkulturböden, Waldstreu und Brachland. Untersuchungen über den optimalen Bodentyp zeigten, daß Lehmböden am günstigsten, reine Sand- und Tonböden am wenigsten geeignet sind, während toniger Lehm und sandiger Ton dazwischen liegen. Es wird geschlossen daß die günstigsten Bodeneigenschaften für Symphyliden in hohem Gehalt an organischer Substanz, guter Feuchtigkeitskapazität und der Fähigkeit zur Verfestigung und Hohlraumbildung bestehen.

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BOOK REVIEW

O. Pflugfelder: **Entwicklungsphysiologie der Insekten.** Akad. Verl. Gesellsch. Leipzig, 2nd Edition 1958, 490 pp., 136 fig.

Six years after the appearance of the first issue, a second edition of Pflugfelder's monograph on insect growth has been necessary. The author has taken this opportunity to revise the book thoroughly, bringing it up-to-date and rendering it still more valuable than it already was before. To me, the greatest value of Pflugfelder's Entwicklungsphysiologie is that it stresses the continuity of embryonic and postembryonic development and moreover places our knowledge of insect metamorphosis amidst classical developmental physiology. In this respect it has no equal. Moreover, it contains a well-ordered, encyclopedic amount of factual evidence and detailed methods.

Though with hesitation in view of so highly esteemed a treatise, some criticisms may perhaps be made.

That cholinesterase would be an essential enzyme to metamorphosis (p. 171) is either an obvious truth (as it is essential to nerve action in general) or a misinterpretation of van der Kloot's finding that cholinesterase activity is greatly suppressed in the brain of diapausing *Hyalophora cecropia.*

I cannot see how the Gomori technique has contributed to an understanding of the relations between the neurosecretory cells and the corpora allata (p. 264), and the author fails to give evidence to support his statement.

The nomenclature given for the groups of glandular cells with large, lobed nuclei involved in the production of the moulting hormone is somewhat erratic. Thus, on p. 272, only the names peritracheal and pericardial glands are mentioned as a component of Weismann's Ring in Brachycera, on p. 380 peritracheal glands and pericardial cells are mentioned as synonyms of the prothoracic glands. As the term prothoracic glands has spread widely in Anglo-saxon physiological literature, it would in my opinion have been of advantage to mention this term at the former place. The use of the name pericardial cells as a synonym is surprising, as these cells greatly differ from the pericardial or prothoracic glands, a fact clearly stated by the author himself (p. 355).

The total revision of the book has inevitably led to considerable extension. It has grown from 332 to 490 pages, and 16 figures have been added.

J. de Wilde

DATE DUE